Docket No.: 134391.00114

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Per Holm *et al.*

Application No.: 10/569,862 Confirmation No.: 2548

Filed: June 13, 2006 Art Unit: 1618

For: MODIFIED RELEASE COMPOSITIONS COMPRISING TACROLIMUS Examiner: M. P. Young

DECLARATION OF NIKOLAJ SKAK

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

I, Nikolaj Skak, hereby declare as follows:

1. I am a citizen of Denmark and over 21 years of age. I have been a Principal Scientist at LifeCycle Pharma A/S (the assignee of the above-identified patent application), located in Hørsholm, Denmark, since February 2010. I have over 7 years of industrial experience in the field of pharmaceutical development and control of pharmaceutical formulations. I began as an Associate Scientist at LifeCycle Pharma A/S in 2003 and was promoted to Research Scientist, and then Senior Research Scientist before becoming a Principal Scientist. I received a M.Sc. in Pharmacy (2003) from The Pharmaceutical University of Denmark (now the University of Copenhagen and previously the Royal Danish School of Pharmacy).

2. The following experiments were performed at LifeCycle Pharma A/S. These experiments repeat the preparation process described in Koretke (International Patent Publication

No. WO 01/95939) but use the drug tacrolimus monohydrate instead of the quinoline compound described in Koretke ((S)-(-)-N-(α-ethylbenzyl)-3-hydroxy-2-phenylquinolin-4-carboxamide).[1]

A. Determination of Heating Time Necessary to Reach 126° C in PEG/Poloxamer Mixture

3. Koretke teaches "melting the drug, the polyethylene glycol [PEG] and the poloxamer surfactant together, with mixing, to form a homogeneous melt mixture" (Koretke, p. 6, lines 25-27). In the sole example in Koretke, the "[m]elt temperatures were above that of the melting point of the free base drug component" (Koretke, p. 7, lines 12-13). The melting point of tacrolimus monohydrate is ~126-130° C. Therefore, in order to ensure melting of the tacrolimus, the mixture of tacrolimus, PEG, and poloxamer should be heated until it reaches at least 126° C.

4. In order to determine the appropriate heating time, a 2 g mixture of PEG 6000 and poloxamer 188 (at a weight ratio of 15:1)[2] was heated at 126° C (the lowest end of the melting point range of ~126-130° C for tacrolimus monohydrate) with stirring. The temperature of the mixture was periodically monitored. The results are shown in Table 1 below.

Table 1

Time (min:sec)	Temperature (°C)
5:00	98.8
5:05	100
6:09	110
8:10	120
10:00	124.5
12:23	126

[1] *See* Koretke, p. 5, lines 32-36, p. 6, lines 4-6 and 25-29, and p. 7, lines 3-38.
[2] The 15:1 weight ratio is the only preferred weight ratio of PEG to poloxamer taught by Koretke (Koretke, p. 7, lines 3-5). Furthermore, PEG 6000 and poloxamer 188 are preferred components according to Koretke (Koretke, p. 5, lines 33-36, and p. 6, lines 4-6).

5. The temperature of the PEG / poloxamer mixture reached 126° C after approximately 12½ minutes of heating. A heating time of 15 minutes was selected for the experiments described below to ensure that the melting point of tacrolimus is reached in the tacrolimus/PEG/poloxamer mixture.

B. Preparation of the Solid Dispersion

6. PEG6000, poloxamer 188 and tacrolimus monohydrate (2 g in total at a weight ratio of 15 : 1 : 4) were weighed into vials. The tacrolimus monohydrate used had a purity of ≥ 98% and contained less than 0.5% by weight of any single impurity. The components were mixed together and heated with stirring to 126° C for the longer of 15 minutes or until a homogeneous melt mixture was formed and confirmed by visual inspection. After heating, the mixture was then allowed to cool for at least 3 hours at room temperature in a desiccator.

7. The amount of tacrolimus remaining in the mixture was measured by HPLC (Zorbax SB-Phenyl column, Mobile Phase A, 30 min run time, isocratic flow, flow rate 0.5mL/min, injection volume 10 µL, detection UV 200nm to 300nm using a DAD, column temperature 45°C, auto sampler temperature 5°C, wash vial Mobile Phase A) relative to Tacrolimus Reference Standard RS 1_1 (retention time ratio of sample solution to reference solution is 0.98-1.02) and calculated as:

$$\% \text{ Assay} = (A_{sample}/A_{reference}) \times (W_{reference} \times P/V_{reference}) \times (V_{sample}/W_{sample}) \times 100 \times 0.978$$

(A is peak area in the sample/RS1_1 chromatogram; W_{ref} is weight of RS1_1; W_{sample} is weight of sample; V_{ref} is volume of RS1_1; V_{sample} is volume of sample; P is purity of RS1_1; 0.978 converts from the monohydrate to the anhydrate).

8. Also, the amount of each of three decomposition products of tacrolimus (namely, the C4-epimer diene, the C8-epimer, and the diene) were measured as described and calculated as

$$\% \text{ Related Substance} = A_{decomposition}/A_{total} \times 100\%$$

(A_{decomp} is area of detected known substance in sample chromatogram; A_{total} is total area of tacrolimus and Related Substances). The chemical structure of the C8-epimer is provided in *Journal of Natural Products*, 2010, Apr 23:73(4):776-79. The chemical structure of the diene is provided in *Pharmacopeial Forum*, 2009, Vol. 35(2). The chemical structure of the C4-epimer (without stereoisomerism) is provided in *Chromatographia*, 2005, Vol. 40 (5/6), p- 253-58 in Table 1, Abbreviated No. XII.

9. This experiment was repeated three times. Each sample was analyzed twice. The results of these experiments are provided in table 2 below.

Table 2

Experiment No.	Analysis No.	Average % tacrolimus (assay)	C4-epimer diene	C8-epimer	Diene
1	A	86.04	0.17	2.47	0.14
	B	86.08	0.16	2.43	0.09
2	A	85.73	0.13	2.24	0.13
	B	85.36	0.16	2.42	0.12
3	A	86.18	0.16	2.47	0.1
	B	86.17	0.14	2.25	0.12
Average		85.93	0.15	2.38	0.12

10. The mixtures produced in experiment numbers 1, 2, and 3 are not pharmaceutically acceptable as they include more than 0.5% of the C8-epimer impurity. 2.24 to 2.47% of the C8-

epimer was identified in each sample. This is above the 0.5% limit for a single degradation product set forth in the International Conference on Harmonisation (ICH) guidelines entitled Topic Q 3 B (R2), Impurities in New Drug Products (Exhibit A, p. 9) (available from the European Medicines Agency (EMA)).[3] Generally, tacrolimus mixtures containing greater than 0.5% of a degradation product are not considered pharmaceutically acceptable, unless additional studies have been performed and establish the safety of the degradation product at the elevated levels observed (Exhibit A, p. 7). To my knowledge, neither the U.S. Food and Drug Administration nor the European Medicines Agency has found a tacrolimus product containing 2.4% or more of the C8-epimer to be safe based on such studies. Accordingly, such a product is not considered pharmaceutically acceptable. Because of the high level of degradation product and double digit reduction in tacrolimus, a skilled artisan would not consider the Koretke process viable for making pharmaceutical formulations of tacrolimus.

11. I hereby declare that all statements made herein of my own knowledge are true and that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

_____ _____

Date Nikolaj Skak

[3] The degradation product limit set forth in the ICH guidelines depends on the maximum daily dosage of the drug. The daily dosage range for tacrolimus is generally 1 mg to 20 mg. The recommended initial daily dosage of Prograf for adult kidney transplant patients (in combination with azathioprine) is 0.2 mg/kg/day. *See* Exhibit B (p. 30). Assuming the average adult weighs 70 kg, the initial daily dosage of Prograf would be 14 mg. For a maximum daily dose ranging from 10 mg to 100 mg, the ICH limit is 0.5% for a single degradation product. *See* Exhibit A, p. 9.

Exhibit A

European Medicines Agency

June 2006
CPMP/ICH/2738/99

ICH Topic Q 3 B (R2)
Impurities in New Drug Products

Step 5

NOTE FOR GUIDANCE ON IMPURITIES IN NEW DRUG PRODUCTS
(CPMP/ICH/2738/99)

TRANSMISSION TO CHMP	November 1999
TRANSMISSION TO INTERESTED PARTIES	November 1999
RELEASE FOR CONSULTATION	November 1999
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APPROVAL BY CPMP	February 2003
DATE FOR COMING INTO OPERATION	August 2003
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IMPURITIES IN NEW DRUG PRODUCTS

I. INTRODUCTION

1.1 Objective of the guideline

This document provides guidance for registration applications on the content and qualification of impurities in new drug products produced from chemically synthesised new drug substances not previously registered in a region or member state.

1.2 Background

This guideline is complementary to the ICH Q3A(R) guideline "Impurities in New Drug Substances", which should be consulted for basic principles. The ICH Q3C guideline "Residual Solvents" should also be consulted, if appropriate.

1.3 Scope of the guideline

This guideline addresses only those impurities in new drug products classified as degradation products of the drug substance or reaction products of the drug substance with an excipient and/or immediate container closure system (collectively referred to as "degradation products" in this guideline). Generally, impurities present in the new drug substance need not be monitored or specified in the new drug product unless they are also degradation products (see ICH Q6A guideline on specifications).

Impurities arising from excipients present in the new drug product or extracted or leached from the container closure system are not covered by this guideline. This guideline also does not apply to new drug products used during the clinical research stages of development. The following types of products are not covered in this guideline: biological/biotechnological products, peptides, oligonucleotides, radiopharmaceuticals, fermentation products and semi-synthetic products derived therefrom, herbal products, and crude products of animal or plant origin. Also excluded from this document are: (1) extraneous contaminants that should not occur in new drug products and are more appropriately addressed as good manufacturing practice (GMP) issues, (2) polymorphic forms, and (3) enantiomeric impurities.

II. RATIONALE FOR THE REPORTING AND CONTROL OF DEGRADATION PRODUCTS

The applicant should summarise the degradation products observed during manufacture and/or stability studies of the new drug product. This summary should be based on sound scientific appraisal of potential degradation pathways in the new drug product and impurities arising from the interaction with excipients and/or the immediate container closure system. In addition, the applicant should summarise any laboratory studies conducted to detect degradation products in the new drug product. This summary should also include test results of batches manufactured during the development process and batches representative of the proposed commercial process. A rationale should be provided for exclusion of those impurities that are not degradation products (e.g., process impurities from the drug substance and impurities arising from excipients). The impurity profiles of the batches representative of the proposed commercial process should be compared with the profiles of batches used in development and any differences discussed.

Any degradation product observed in stability studies conducted at the recommended storage condition should be identified when present at a level greater than (>) the identification thresholds given in Attachment 1. When identification of a degradation product is not feasible, a summary of the laboratory studies demonstrating the unsuccessful efforts to identify it should be included in the registration application.
Degradation products present at a level of not more than (≤) the identification threshold generally would not need to be identified. However, analytical procedures should be developed for those degradation products that are suspected to be unusually potent, producing toxic or significant pharmacological effects at levels not more than (≤) the identification threshold. In unusual circumstances, technical factors (e.g., manufacturing capability, a low drug substance to excipient ratio, or the use of excipients that are crude products of animal or plant origin) can be considered as part of the justification for selection of alternative thresholds based upon manufacturing experience with the proposed commercial process.

III. ANALYTICAL PROCEDURES

The registration application should include documented evidence that the analytical procedures have been validated and are suitable for the detection and quantitation of degradation products (see ICH Q2A and Q2B guidelines on analytical validation). In particular, analytical procedures should be validated to demonstrate specificity for the specified and unspecified degradation products. As appropriate, this validation should include samples stored under relevant stress conditions: light, heat, humidity, acid/base hydrolysis, and oxidation. When an analytical procedure reveals the presence of other peaks in addition to those of the degradation products (e.g., the drug substance, impurities arising from the synthesis of the drug substance, excipients and impurities arising from the excipients), these peaks should be labeled in the chromatograms and their origin(s) discussed in the validation documentation.

The quantitation limit for the analytical procedure should be not more than (≤) the reporting threshold.

Degradation product levels can be measured by a variety of techniques, including those that compare an analytical response for a degradation product to that of an appropriate reference standard or to the response of the new drug substance itself. Reference standards used in the analytical procedures for control of degradation products should be evaluated and characterised according to their intended uses. The drug substance can be used to estimate the levels of degradation products. In cases where the response factors are not close, this practice can still be used if a correction factor is applied or the degradation products are, in fact, being overestimated. Acceptance criteria and analytical procedures, used to estimate identified or unidentified degradation products, are often based on analytical assumptions (e.g., equivalent detector response). These assumptions should be discussed in the registration application.

Differences between the analytical procedures used during development and those proposed for the commercial product should also be discussed.

IV REPORTING DEGRADATION PRODUCTS CONTENT OF BATCHES

Analytical results should be provided in the registration application for all relevant batches of the new drug product used for clinical, safety, and stability testing, as well as batches that are representative of the proposed commercial process. Quantitative results should be presented numerically, and not in general terms such as "complies", "meets limit" etc. Any degradation product at a level greater than (>) the reporting threshold (see Attachment 1), and total degradation products observed in the relevant batches of the new drug product, should be reported with the analytical procedures indicated. Below 1.0%, the results should be reported to the number of decimal places (e.g., 0.06%) in the applicable reporting threshold; at and above 1.0%, the results should be reported to one decimal place (e.g., 1.3%). Results should be rounded using conventional rules (see Attachment 2). A tabulation (e.g., spreadsheet) of the data is recommended. Degradation products should be designated by code number or by an appropriate descriptor, e.g., retention time. If a higher reporting threshold is proposed, it should be fully justified. All degradation products at a level greater than (>) the reporting threshold should be summed and reported as total degradation products.
Chromatograms with peaks labelled (or equivalent data if other analytical procedures are used) from representative batches, including chromatograms from analytical procedure validation studies and from long-term and accelerated stability studies, should be provided. The applicant should ensure that complete degradation product profiles (e.g., chromatograms) of individual batches are available, if requested.

For each batch of the new drug product described in the registration application, the documentation should include:

- Batch identity, strength, and size
- Date of manufacture
- Site of manufacture
- Manufacturing process
- Immediate container closure
- Degradation product content, individual and total
- Use of batch (e.g., clinical studies, stability studies)
- Reference to analytical procedure used
- Batch number of the drug substance used in the new drug product
- Storage conditions for stability studies

V. LISTING OF DEGRADATION PRODUCTS IN SPECIFICATIONS

The specification for a new drug product should include a list of degradation products expected to occur during manufacture of the commercial product and under recommended storage conditions. Stability studies, knowledge of degradation pathways, product development studies, and laboratory studies should be used to characterise the degradation profile. The selection of degradation products in the new drug product specification should be based on the degradation products found in batches manufactured by the proposed commercial process. Those individual degradation products with specific acceptance criteria included in the specification for the new drug product are referred to as "specified degradation products" in this guideline. Specified degradation products can be identified or unidentified. A rationale for the inclusion or exclusion of degradation products in the specification should be presented. This rationale should include a discussion of the degradation profiles observed

in the safety and clinical development batches and in stability studies, together with a consideration of the degradation profile of batches manufactured by the proposed commercial process. Specified identified degradation products should be included along with specified unidentified degradation products estimated to be present at a level greater than (>) the identification threshold given in Attachment 1. For degradation products known to be unusually potent or to produce toxic or unexpected pharmacological effects, the quantitation/detection limit of the analytical procedures should be commensurate with the level at which the degradation products should be controlled. For unidentified degradation products, the procedure used and assumptions made in establishing the level of the degradation product should be clearly stated. Specified unidentified degradation products should be referred to by an appropriate qualitative analytical descriptive label (e.g., "unidentified A", "unidentified with relative retention of 0.9"). A general acceptance criterion of not more than (≤) the identification threshold (Attachment 1) for any unspecified degradation product and an acceptance criterion for total degradation products should also be included.
For a given degradation product, its acceptance criterion should be established by taking into account its acceptance criterion in the drug substance (if applicable), its qualified level, its increase during stability studies, and the proposed shelf life and recommended storage conditions for the new drug product. Furthermore, each acceptance criterion should be set no higher than the qualified level of the given degradation product.

Where there is no safety concern, degradation product acceptance criteria should be based on data generated from batches of the new drug product manufactured by the proposed commercial process, allowing sufficient latitude to deal with normal manufacturing and analytical variation and the stability characteristics of the new drug product. Although normal manufacturing variations are expected, significant variation in batch-to-batch degradation product levels can indicate that the manufacturing process of the new drug product is not adequately controlled and validated (see ICH Q6A guideline on specifications, decision tree #2, for establishing an acceptance criterion for a specified degradation product in a new drug product).

In this guideline, the use of two decimal places for thresholds (See Attachment 1) does not necessarily indicate the precision of the acceptance criteria for specified degradation products and total degradation products.

In summary, the new drug product specification should include, where applicable, the following list of degradation products:

- Each specified identified degradation product
- Each specified unidentified degradation product
- Any unspecified degradation product with an acceptance criterion of not more than (≤) the identification threshold
- Total degradation products.

VI QUALIFICATION OF DEGRADATION PRODUCTS

Qualification is the process of acquiring and evaluating data that establishes the biological safety of an individual degradation product or a given degradation profile at the level(s) specified. The applicant should provide a rationale for establishing degradation product acceptance criteria that includes safety considerations. The level of any degradation product present in a new drug product that has been adequately tested in safety and/or clinical studies

would be considered qualified. Therefore, it is useful to include any available information on the actual content of degradation products in the relevant batches at the time of use in safety and/or clinical studies. Degradation products that are also significant metabolites present in animal and/or human studies are generally considered qualified. Degradation products could be considered qualified at levels higher than those administered in safety studies based on a comparison between actual doses given in the safety studies and the intended dose of the new drug product. Justification of such higher levels should include consideration of factors such as: (1) the amount of degradation product administered in previous safety and/or clinical studies and found to be safe; (2) the increase in the amount of the degradation product; and (3) other safety factors, as appropriate.

If the qualification thresholds given in Attachment 1 are exceeded and data are unavailable to qualify the proposed acceptance criterion of a degradation product, additional studies to obtain such data can be appropriate (see Attachment 3).

Higher or lower thresholds for qualification of degradation products can be appropriate for some individual new drug products based on scientific rationale and level of concern, including drug class effects and clinical experience. For example, qualification can be especially important when there is evidence that such degradation products in certain new drug products or therapeutic classes have previously been associated with adverse reactions in patients. In these instances, a lower qualification threshold can be appropriate. Conversely, a higher qualification threshold can be appropriate for individual new drug products when the level of concern for safety is less than usual based on similar considerations (e.g., patient population, drug class effects, and clinical considerations). Proposals for alternative thresholds would be considered on a case-by-case basis.

The "Decision Tree for Identification and Qualification of a Degradation Product" (Attachment 3) describes considerations for the qualification of degradation products when thresholds are exceeded. In some cases, reducing the level of degradation product (e.g., use of a more protective container closure or modified storage conditions) to not more than (≤) the threshold can be simpler than providing safety data. Alternatively, adequate data could be available in the scientific literature to qualify a degradation product. If neither is the case, additional safety testing should be considered. The studies considered appropriate to qualify a degradation product will depend on a number of factors, including the patient population, daily dose, and route and duration of new drug product administration. Such studies can be conducted on the new drug product or substance containing the degradation products to be controlled, although studies using isolated degradation products can sometimes be appropriate.

Although this guideline is not intended to apply during the clinical research stage of development, in the later stages of development the thresholds in this guideline can be useful in evaluating new degradation products observed in new drug product batches prepared by the proposed commercial process. Any new degradation product observed in later stages of development should be identified (see the "Decision Tree for Identification and Qualification of a Degradation Product" in Attachment 3) if its level is greater than (>) the identification threshold given in Attachment 1. Similarly, qualification of the degradation product should be considered if its level is greater than (>) the qualification threshold given in Attachment 1.

Safety studies should provide a comparison of results of safety testing of the new drug product or drug substance containing a representative level of the degradation product with previously qualified material, although studies using the isolated degradation products can also be considered.

VII GLOSSARY

Degradation Product: An impurity resulting from a chemical change in the drug substance brought about during manufacture and/or storage of the new drug product by the effect of, for example, light, temperature, pH, water, or by reaction with an excipient and/or the immediate container closure system.

Degradation Profile: A description of the degradation products observed in the drug substance or drug product.

Development Studies: Studies conducted to scale-up, optimise, and validate the manufacturing process for a drug product.

Identification Threshold: A limit above (>) which a degradation product should be identified.

Identified Degradation Product: A degradation product for which a structural characterisation has been achieved.

Impurity: Any component of the new drug product that is not the drug substance or an excipient in the drug product.

Impurity Profile: A description of the identified and unidentified impurities present in a drug product.

New Drug Substance: The designated therapeutic moiety that has not been previously registered in a region or member state (also referred to as a new molecular entity or new chemical entity). It can be a complex, simple ester, or salt of a previously approved substance.

Qualification: The process of acquiring and evaluating data that establishes the biological safety of an individual degradation product or a given degradation profile at the level(s) specified.

Qualification Threshold: A limit above (>) which a degradation product should be qualified.

Reporting Threshold: A limit above (>) which a degradation product should be reported.

Specified Degradation Product: A degradation product that is individually listed and limited with a specific acceptance criterion in the new drug product specification. A specified degradation product can be either identified or unidentified.

Unidentified Degradation Product: A degradation product for which a structural characterisation has not been achieved and that is defined solely by qualitative analytical properties (e.g., chromatographic retention time).

Unspecified Degradation Product: A degradation product that is limited by a general acceptance criterion, but not individually listed with its own specific acceptance criterion, in the new drug product specification.

Attachment 1: Thresholds for Degradation Products in New Drug Products

Reporting Thresholds

Maximum Daily Dose[1]	Threshold[2,3]
≤ 1 g	0.1%
> 1 g	0.05%

Identification Thresholds

Maximum Daily Dose[1]	Threshold[2, 3]
< 1 mg	1.0% or 5 µg TDI, whichever is lower
1 mg - 10 mg	0.5% or 20 µg TDI, whichever is lower
>10 mg - 2 g	0.2% or 2 mg TDI, whichever is lower
> 2 g	0.10%

Qualification Thresholds

Maximum Daily Dose[1]	Threshold[2,3]
< 10 mg	1.0% or 50 µg TDI, whichever is lower
10 mg - 100 mg	0.5% or 200 µg TDI, whichever is lower
>100 mg - 2 g	0.2% or 3 mg TDI, whichever is lower
> 2 g	0.15%

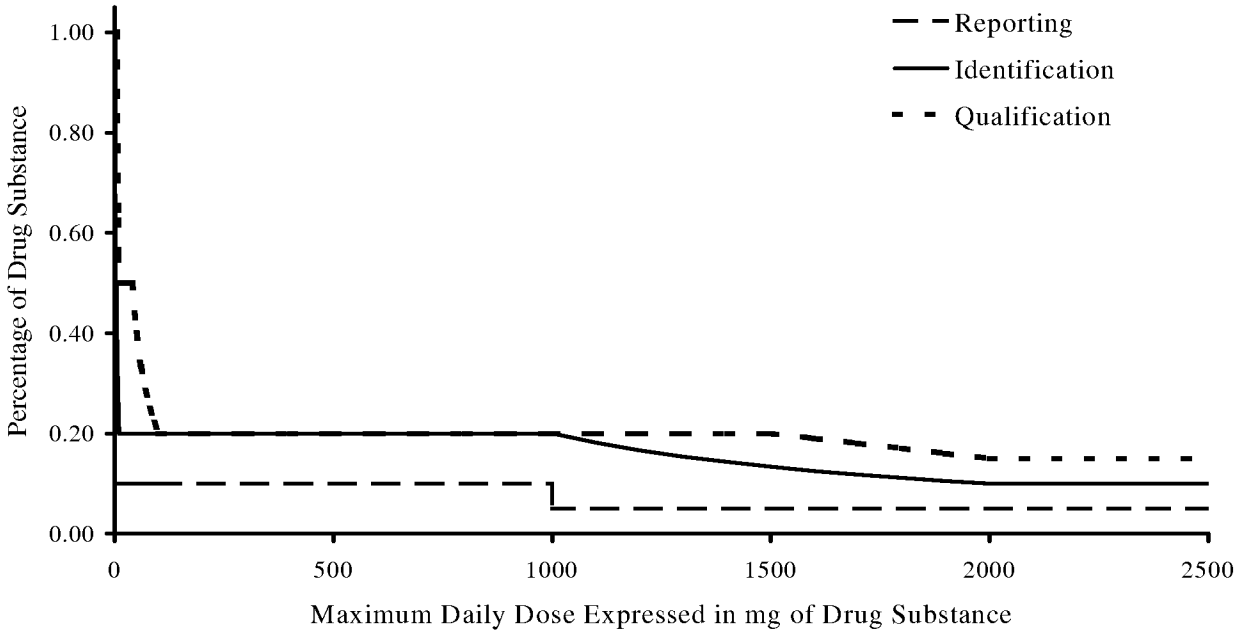
Notes on Attachment 1

1 The amount of drug substance administered per day

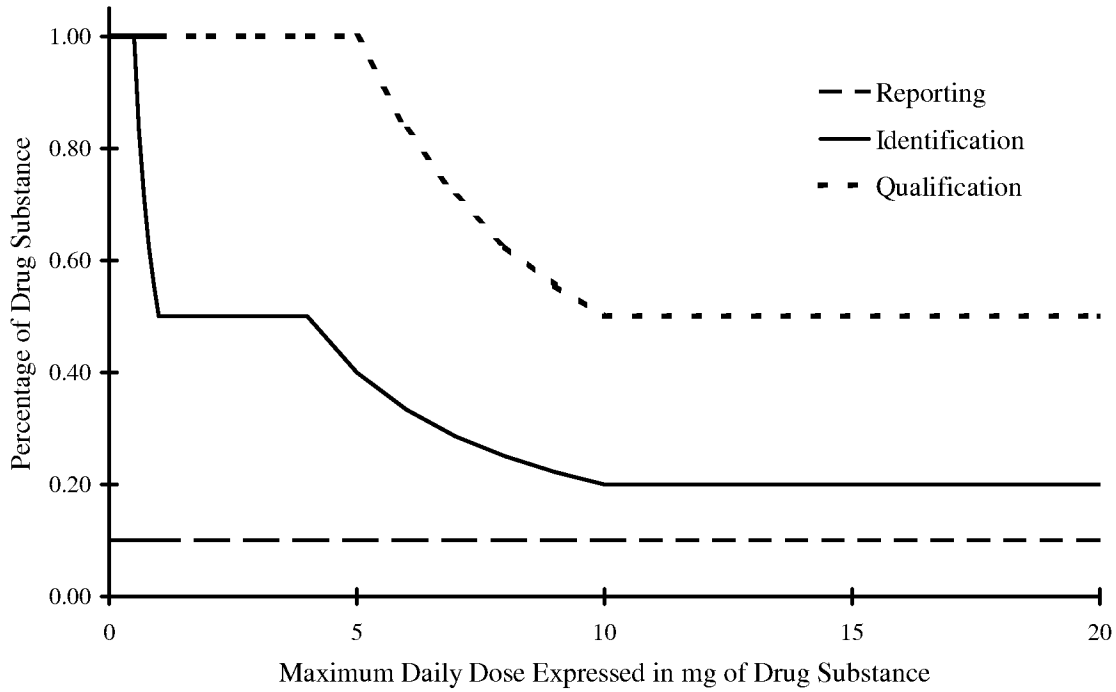
2 Thresholds for degradation products are expressed either as a percentage of the drug substance or as total daily intake (TDI) of the degradation product. Lower thresholds can be appropriate if the degradation product is unusually toxic.

3 Higher thresholds should be scientifically justified.

Illustration of Thresholds for Reporting, Identification, and Qualification of Degradation Products in New Drug Products as a Function of Maximum Daily Dose[1]



Expanded Scale:



1 Note: Actual threshold values should be taken from the preceding table in this attachment.

Attachment 2: Illustration of Reporting Degradation product Results for Identification and Qualification in an Application

The attachment is only illustrative and is not intended to serve as a template how results on degradation products should be presented in an application file. Normally raw data are not provided.

Example 1: 50 mg Maximum Daily Dose

Reporting threshold: 0.1%
Identification threshold: 0.2%
Qualification threshold: 200 µg

'Raw' Result (%)	Reported Result (%) (Reporting Threshold = 0.1%)	Total Daily Intake (TDI) of the Degradation Product (rounded result in µg)	Action	
			Identification Threshold 0.2% exceeded?	Qualification Threshold 200 µg TDI exceeded?
0.04	Not reported	20	None	None
0.2143	0.2	100	None	None
0.349	0.3[1]	150	Yes	None[1]
0.550	0.6[1]	300	Yes	Yes[1]

Example 2: 1.9 gram Maximum Daily Dose

Reporting threshold: 0.05%
Identification threshold: 2 mg
Qualification threshold: 3 mg

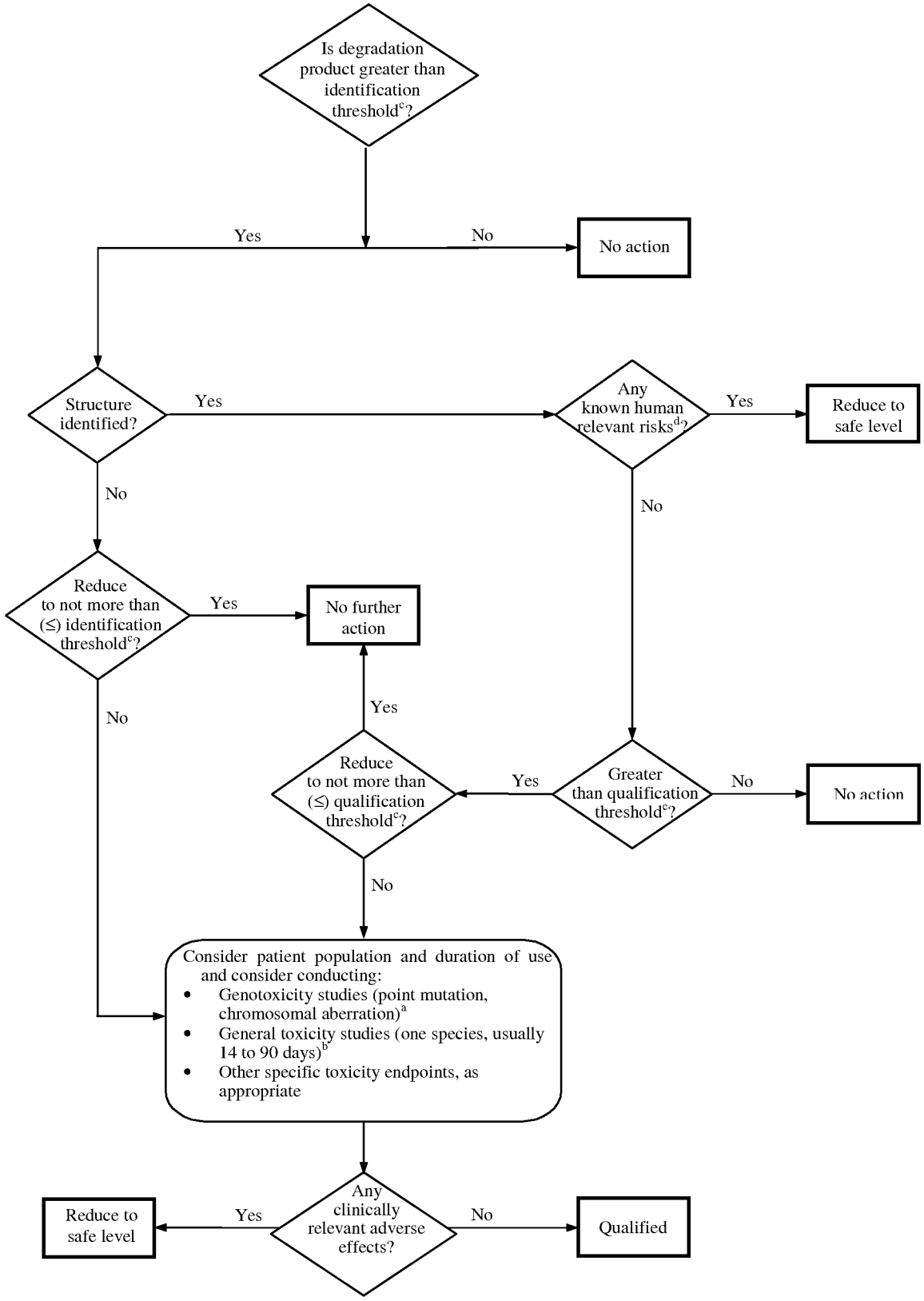
'Raw' Result (%)	Reported Result (%) (Reporting Threshold = 0.05%)	Total Daily Intake (TDI) of the Degradation Product (rounded result in mg)	Action	
			Identification Threshold 2 mg TDI exceeded?	Qualification Threshold 3 mg TDI exceeded?
0.049	Not reported	1	None	None
0.079	0.08	2	None	None
0.183	0.18[1]	3	Yes	None[1, 2]
0.192	0.19[1]	4	Yes	Yes[1]

Notes on attachment 2

1 After identification, if the response factor is determined to differ significantly from the original assumptions, it can be appropriate to re-measure the actual amount of the degradation product present and re-evaluate against the qualification threshold (see Attachment 1).

2 To verify if a threshold is exceeded, a reported result has to be evaluated against the thresholds as follows: when the threshold is described in %, the reported result rounded to the same decimal place as the threshold should be compared directly to the threshold. When the threshold is described in TDI, the reported result should be converted to TDI, rounded to the same decimal place as the threshold and compared to the threshold e.g. an amount of 0.18% degradation level corresponds to a TDI of 3.4 mg impurity (absolute amount) which is then rounded down to 3 mg; so the qualification threshold expressed in TDI (3 mg) is not exceeded.

Attachment 3: Decision Tree for Identification and Qualification of a Degradation Product



Notes on Attachment 3

a) If considered desirable, a minimum screen (e.g., genotoxic potential), should be conducted.
A study to detect point mutations and one to detect chromosomal aberrations, both in vitro, are considered an appropriate minimum screen.

b) If general toxicity studies are desirable, one or more studies should be designed to allow comparison of unqualified to qualified material. The study duration should be based on available relevant information and performed in the species most likely to maximise the potential to detect the toxicity of a degradation product. On a case-by-case basis, single-dose studies can be appropriate, especially for single-dose drugs. In general, a minimum duration of 14 days and a maximum duration of 90 days would be considered appropriate.

c) Lower thresholds can be appropriate if the degradation product is unusually toxic.

d) For example, do known safety data for this degradation product or its structural class preclude human exposure at the concentration present?

PROGRAF®
tacrolimus capsules
tacrolimus injection (for intravenous infusion only)

Revised: August 2009

WARNING

Increased susceptibility to infection and the possible development of lymphoma may result from immunosuppression. Only physicians experienced in immunosuppressive therapy and management of organ transplant patients should prescribe Prograf. Patients receiving the drug should be managed in facilities equipped and staffed with adequate laboratory and supportive medical resources. The physician responsible for maintenance therapy should have complete information requisite for the follow-up of the patient.

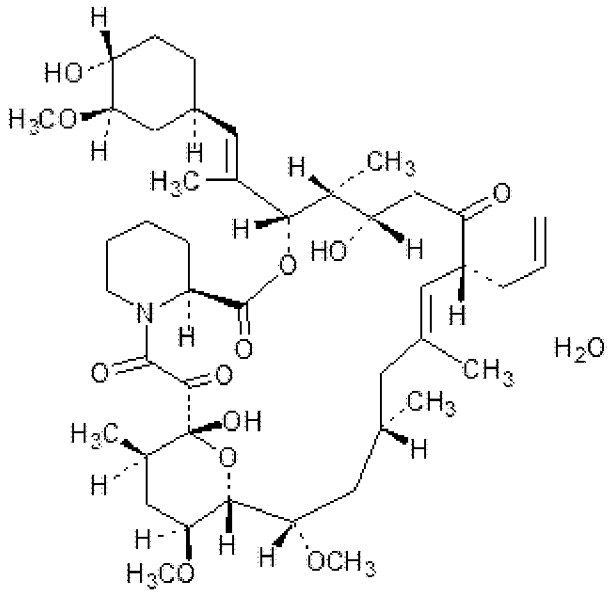
DESCRIPTION

Prograf is available for oral administration as capsules (tacrolimus capsules) containing the equivalent of 0.5 mg, 1 mg or 5 mg of anhydrous tacrolimus. Inactive ingredients include lactose, hydroxypropyl methylcellulose, croscarmellose sodium, and magnesium stearate. The 0.5 mg capsule shell contains gelatin, titanium dioxide and ferric oxide, the 1 mg capsule shell contains gelatin and titanium dioxide, and the 5 mg capsule shell contains gelatin, titanium dioxide and ferric oxide.

Prograf is also available as a sterile solution (tacrolimus injection) containing the equivalent of 5 mg anhydrous tacrolimus in 1 mL for administration by intravenous infusion only. Each mL contains polyoxyl 60 hydrogenated castor oil (HCO-60), 200 mg, and dehydrated alcohol, USP, 80.0% v/v. Prograf injection must be diluted with 0.9% Sodium Chloride Injection or 5% Dextrose Injection before use.

Tacrolimus, previously known as FK506, is the active ingredient in Prograf. Tacrolimus is a macrolide immunosuppressant produced by *Streptomyces tsukubaensis*. Chemically, tacrolimus is designated as [3*S*-[3*R**[*E*(1*S**,3*S**,4*S**)], 4*S**,5*R**,8*S**,9*E*,12*R**,14*R**,15*S**,16*R**,18*S**,19*S**,26a*R**]] - 5,6,8,11,12,13,14,15,16,17,18,19,24,25,26,26a-hexadecahydro-5,19-dihydroxy-3-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylethenyl]-14,16-dimethoxy-4,10,12,18-tetramethyl-8-(2-propenyl)-15,19-epoxy-3H-pyrido[2,1-*c*][1,4] oxaazacyclotricosine-1,7,20,21(4H,23H)-tetrone, monohydrate.

The chemical structure of tacrolimus is:



Tacrolimus has an empirical formula of $C_{44}H_{69}NO_{12}•H_2O$ and a formula weight of 822.03. Tacrolimus appears as white crystals or crystalline powder. It is practically insoluble in water, freely soluble in ethanol, and very soluble in methanol and chloroform.

CLINICAL PHARMACOLOGY

Mechanism of Action

Tacrolimus prolongs the survival of the host and transplanted graft in animal transplant models of liver, kidney, heart, bone marrow, small bowel and pancreas, lung and trachea, skin, cornea, and limb.

In animals, tacrolimus has been demonstrated to suppress some humoral immunity and, to a greater extent, cell-mediated reactions such as allograft rejection, delayed type hypersensitivity, collagen-induced arthritis, experimental allergic encephalomyelitis, and graft versus host disease.

Tacrolimus inhibits T-lymphocyte activation, although the exact mechanism of action is not known. Experimental evidence suggests that tacrolimus binds to an intracellular protein, FKBP-12. A complex of tacrolimus-FKBP-12, calcium, calmodulin, and calcineurin is then formed and the phosphatase activity of calcineurin inhibited. This effect may prevent the dephosphorylation and translocation of nuclear factor of activated T-cells (NF-AT), a nuclear component thought to initiate gene transcription for the formation of lymphokines (such as interleukin-2, gamma interferon). The net result is the inhibition of T-lymphocyte activation (i.e., immunosuppression).

Pharmacokinetics

Tacrolimus activity is primarily due to the parent drug. The pharmacokinetic parameters (mean±S.D.) of tacrolimus have been determined following intravenous (IV) and/or oral (PO) administration in healthy volunteers, and in kidney transplant, liver transplant, and heart transplant patients. (See table below.)

Population	**N**	**Route (Dose)**	Parameters					
			C_{max} (ng/mL)	**T_{max} (hr)**	**AUC (ng•hr/mL)**	**$t_{1/2}$ (hr)**	**CI (L/hr/kg)**	**V (L/kg)**

Healthy Volunteers	8	IV (0.025 mg/kg/4hr)	—	—	598‡ ± 125	34.2 ± 7.7	0.040 ± 0.009	1.91 ± 0.31
	16	PO (5 mg)	29.7 ± 7.2	1.6 ± 0.7	243§ ± 73	34.8 ± 11.4	0.041† ± 0.008	1.94† ± 0.53
Kidney Transplant Pts	26	IV (0.02 mg/kg/12 hr)	—	—	294¶ ± 262	18.8 ± 16.7	0.083 ± 0.050	1.41 ± 0.66
		PO (0.2 mg/kg/day)	19.2 ± 10.3	3.0	203¶ ± 42	#	#	#
		PO (0.3 mg/kg/day)	24.2 ± 15.8	1.5	288¶ ± 93	#	#	#
Liver Transplant Pts	17	IV (0.05 mg/kg/12 hr)	—	—	3300¶ ± 2130	11.7 ± 3.9	0.053 ± 0.017	0.85 ± 0.30
		PO (0.3 mg/kg/day)	68.5 ± 30.0	2.3 ± 1.5	519¶ ± 179	#	#	#
Heart Transplant Patients	11	IV (0.01 mg/kg/day as a continuous infusion)	—	—	954‖ ±334	23.6 ±9.22	0.051 ±0.015	#
	11	PO (0.075mg/kg/day)***	14.7±7.79	2.1 [0.5-6.0]**	82.7* ±63.2	—	#	#
	14	PO (0.15mg/kg/day)***	24.5± 13.7	1.5 [0.4-4.0]**	142*±116	—	#	#

† Corrected for individual bioavailability; ‡ AUC_{0-120}; § AUC_{0-72}; ¶ AUC_{0-inf}; ‖ AUC_{0-t}; * AUC_{0-12}; ** Median [range]; *** Determined after the first dose; — not applicable; # not available

Due to intersubject variability in tacrolimus pharmacokinetics, individualization of dosing regimen is necessary for optimal therapy. (See DOSAGE AND ADMINISTRATION). Pharmacokinetic data

indicate that whole blood concentrations rather than plasma concentrations serve as the more appropriate sampling compartment to describe tacrolimus pharmacokinetics.

Absorption

Absorption of tacrolimus from the gastrointestinal tract after oral administration is incomplete and variable. The absolute bioavailability of tacrolimus was 17±10% in adult kidney transplant patients (N=26), 22±6% in adult liver transplant patients (N=17), 23±9% in adult heart transplant patients (N=11) and 18±5% in healthy volunteers (N=16).

A single dose study conducted in 32 healthy volunteers established the bioequivalence of the 1 mg and 5 mg capsules. Another single dose study in 32 healthy volunteers established the bioequivalence of the 0.5 mg and 1 mg capsules. Tacrolimus maximum blood concentrations (C_{max}) and area under the curve (AUC) appeared to increase in a dose-proportional fashion in 18 fasted healthy volunteers receiving a single oral dose of 3, 7, and 10 mg.

In 18 kidney transplant patients, tacrolimus trough concentrations from 3 to 30 ng/mL measured at 10-12 hours post-dose (C_{min}) correlated well with the AUC (correlation coefficient 0.93). In 24 liver transplant patients over a concentration range of 10 to 60 ng/mL, the correlation coefficient was 0.94. In 25 heart transplant patients over a concentration range of 2 to 24 ng/mL, the correlation coefficient was 0.89 after an oral dose of 0.075 or 0.15 mg/kg/day at steady-state.

Food Effects

The rate and extent of tacrolimus absorption were greatest under fasted conditions. The presence and composition of food decreased both the rate and extent of tacrolimus absorption when administered to 15 healthy volunteers.

The effect was most pronounced with a high-fat meal (848 kcal, 46% fat): mean AUC and C_{max} were decreased 37% and 77%, respectively; T_{max} was lengthened 5-fold. A high-carbohydrate meal (668 kcal, 85% carbohydrate) decreased mean AUC and mean C_{max} by 28% and 65%, respectively.

In healthy volunteers (N=16), the time of the meal also affected tacrolimus bioavailability. When given immediately following the meal, mean C_{max} was reduced 71%, and mean AUC was reduced 39%, relative to the fasted condition. When administered 1.5 hours following the meal, mean C_{max} was reduced 63%, and mean AUC was reduced 39%, relative to the fasted condition.

In 11 liver transplant patients, Prograf administered 15 minutes after a high fat (400 kcal, 34% fat) breakfast, resulted in decreased AUC (27±18%) and C_{max} (50±19%), as compared to a fasted state.

Distribution

The plasma protein binding of tacrolimus is approximately 99% and is independent of concentration over a range of 5-50 ng/mL. Tacrolimus is bound mainly to albumin and alpha-1-acid glycoprotein, and has a high level of association with erythrocytes. The distribution of tacrolimus between whole blood and plasma depends on several factors, such as hematocrit, temperature at the time of plasma separation, drug concentration, and plasma protein concentration. In a U.S. study, the ratio of whole blood concentration to plasma concentration averaged 35 (range 12 to 67).

Metabolism

Tacrolimus is extensively metabolized by the mixed-function oxidase system, primarily the cytochrome P-450 system (CYP3A). A metabolic pathway leading to the formation of 8 possible metabolites has been proposed. Demethylation and hydroxylation were identified as the primary mechanisms of

biotransformation in vitro. The major metabolite identified in incubations with human liver microsomes is 13-demethyl tacrolimus. In in vitro studies, a 31-demethyl metabolite has been reported to have the same activity as tacrolimus.

Excretion

The mean clearance following IV administration of tacrolimus is 0.040, 0.083, and 0.053, and 0.051 L/hr/kg in healthy volunteers, adult kidney transplant patients, adult liver transplant patients, and adult heart transplant patients, respectively. In man, less than 1% of the dose administered is excreted unchanged in urine.

In a mass balance study of IV administered radiolabeled tacrolimus to 6 healthy volunteers, the mean recovery of radiolabel was 77.8±12.7%. Fecal elimination accounted for 92.4±1.0% and the elimination half-life based on radioactivity was 48.1±15.9 hours whereas it was 43.5±11.6 hours based on tacrolimus concentrations. The mean clearance of radiolabel was 0.029±0.015 L/hr/kg and clearance of tacrolimus was 0.029±0.009 L/hr/kg. When administered PO, the mean recovery of the radiolabel was 94.9±30.7%. Fecal elimination accounted for 92.6±30.7%, urinary elimination accounted for 2.3±1.1% and the elimination half-life based on radioactivity was 31.9±10.5 hours whereas it was 48.4± 12.3 hours based on tacrolimus concentrations. The mean clearance of radiolabel was 0.226±0.116 L/hr/kg and clearance of tacrolimus 0.172± 0.088 L/hr/kg.

Special Populations

Pediatric

Pharmacokinetics of tacrolimus have been studied in liver transplantation patients, 0.7 to 13.2 years of age. Following IV administration of a 0.037 mg/kg/day dose to 12 pediatric patients, mean terminal half-life, volume of distribution and clearance were 11.5±3.8 hours, 2.6±2.1 L/kg and 0.138± 0.071 L/hr/kg, respectively. Following oral administration to 9 patients, mean AUC and C_{max} were 337±167 ng·hr/mL and 48.4±27.9 ng/mL, respectively. The absolute bioavailability was 31±24%.

Whole blood trough concentrations from 31 patients less than 12 years old showed that pediatric patients needed higher doses than adults to achieve similar tacrolimus trough concentrations. (See DOSAGE AND ADMINISTRATION).

Renal and Hepatic Insufficiency

The mean pharmacokinetic parameters for tacrolimus following single administrations to patients with renal and hepatic impairment are given in the following table

Population (No. of Patients)	Dose	AUC_{0-t} (ng·hr/ mL)	$t_{1/2}$ (hr)	V (L/kg)	CI (L/hr/kg)
Renal Impairment (n=12)	0.02 mg/kg/4hr IV	393±123 (t=60 hr)	26.3 ±9.2	1.07 ±0.20	0.038 ±0.014
Mild Hepatic	0.02	367±107	60.6±43.8	3.1±1.6	0.042

Impairment (n=6)	mg/kg/4hr IV	(t=72 hr)	Range: 27.8 – 141		±0.02
	7.7 mg PO	488±320 (t=72 hr)	66.1±44.8 Range: 29.5 – 138	3.7±4.7*	0.034 ±0.019*
Severe Hepatic Impairment (n=6, IV)	0.02 mg/kg/4hr IV (n=2)	762±204 (t=120 hr)	198±158 Range:81-436	3.9±1.0	0.017 ±0.013
	0.01 mg/kg/8hr IV (n=4)	289±117 (t=144 hr)			
(n=5, PO) †	8 mg PO (n=1)	658 (t=120 hr)	119±35 Range: 85-178	3.1±3.4*	0.016 ±0.011*
	5 mg PO (n=4)	533±156 (t=144 hr)			
	4 mg PO (n=1)				

* corrected for bioavailability

† 1 patient did not receive the PO dose

Renal Insufficiency: Tacrolimus pharmacokinetics following a single IV administration were determined in 12 patients (7 not on dialysis and 5 on dialysis, serum creatinine of 3.9±1.6 and 12.0±2.4 mg/dL, respectively) prior to their kidney transplant. The pharmacokinetic parameters obtained were similar for both groups.

The mean clearance of tacrolimus in patients with renal dysfunction was similar to that in normal volunteers (see previous table).

Hepatic Insufficiency: Tacrolimus pharmacokinetics have been determined in six patients with mild hepatic dysfunction (mean Pugh score: 6.2) following single IV and oral administrations. The mean clearance of tacrolimus in patients with mild hepatic dysfunction was not substantially different from that in normal volunteers (see previous table). Tacrolimus pharmacokinetics were studied in 6 patients with severe hepatic dysfunction (mean Pugh score: >10). The mean clearance was substantially lower in patients with severe hepatic dysfunction, irrespective of the route of administration.

Race

A formal study to evaluate the pharmacokinetic disposition of tacrolimus in Black transplant patients has not been conducted. However, a retrospective comparison of Black and Caucasian kidney transplant patients indicated that Black patients required higher tacrolimus doses to attain similar trough concentrations. (See DOSAGE AND ADMINISTRATION.)

Gender

A formal study to evaluate the effect of gender on tacrolimus pharmacokinetics has not been conducted, however, there was no difference in dosing by gender in the kidney transplant trial. A retrospective comparison of pharmacokinetics in healthy volunteers, and in kidney, liver and heart transplant patients indicated no gender-based differences.

CLINICAL STUDIES

Liver Transplantation

The safety and efficacy of Prograf-based immunosuppression following orthotopic liver transplantation were assessed in two prospective, randomized, non-blinded multicenter studies. The active control groups were treated with a cyclosporine-based immunosuppressive regimen. Both studies used concomitant adrenal corticosteroids as part of the immunosuppressive regimens. These studies were designed to evaluate whether the two regimens were therapeutically equivalent, with patient and graft survival at 12 months following transplantation as the primary endpoints. The Prograf-based immunosuppressive regimen was found to be equivalent to the cyclosporine-based immunosuppressive regimens.

In one trial, 529 patients were enrolled at 12 clinical sites in the United States; prior to surgery, 263 were randomized to the Prograf-based immunosuppressive regimen and 266 to a cyclosporine-based immunosuppressive regimen (CBIR). In 10 of the 12 sites, the same CBIR protocol was used, while 2 sites used different control protocols. This trial excluded patients with renal dysfunction, fulminant hepatic failure with Stage IV encephalopathy, and cancers; pediatric patients ($\leq$ 12 years old) were allowed.

In the second trial, 545 patients were enrolled at 8 clinical sites in Europe; prior to surgery, 270 were randomized to the Prograf-based immunosuppressive regimen and 275 to CBIR. In this study, each center used its local standard CBIR protocol in the active-control arm. This trial excluded pediatric patients, but did allow enrollment of subjects with renal dysfunction, fulminant hepatic failure in Stage IV encephalopathy, and cancers other than primary hepatic with metastases.

One-year patient survival and graft survival in the Prograf-based treatment groups were equivalent to those in the CBIR treatment groups in both studies. The overall 1-year patient survival (CBIR and Prograf-based treatment groups combined) was 88% in the U.S. study and 78% in the European study. The overall 1-year graft survival (CBIR and Prograf-based treatment groups combined) was 81% in the U.S. study and 73% in the European study. In both studies, the median time to convert from IV to oral Prograf dosing was 2 days.

Because of the nature of the study design, comparisons of differences in secondary endpoints, such as incidence of acute rejection, refractory rejection or use of OKT3 for steroid-resistant rejection, could not be reliably made.

Kidney Transplantation

Prograf/azathioprine

Prograf-based immunosuppression in conjunction with azathioprine and corticosteroids following kidney transplantation was assessed in a Phase 3 randomized, multicenter, non-blinded, prospective study. There were 412 kidney transplant patients enrolled at 19 clinical sites in the United States. Study therapy was initiated when renal function was stable as indicated by a serum creatinine $\leq$ 4 mg/dL (median of 4 days after transplantation, range 1 to 14 days). Patients less than 6 years of age were excluded.

There were 205 patients randomized to Prograf-based immunosuppression and 207 patients were randomized to cyclosporine-based immunosuppression. All patients received prophylactic induction

therapy consisting of an antilymphocyte antibody preparation, corticosteroids and azathioprine. Overall 1 year patient and graft survival was 96.1% and 89.6%, respectively and was equivalent between treatment arms.

Because of the nature of the study design, comparisons of differences in secondary endpoints, such as incidence of acute rejection, refractory rejection or use of OKT3 for steroid-resistant rejection, could not be reliably made.

Prograf/mycophenolate mofetil (MMF)

Prograf-based immunosuppression in conjunction with MMF, corticosteroids, and induction has been studied. In a randomized, open-label, multi-center trial (Study 1), 1589 kidney transplant patients received Prograf (Group C, n=401), sirolimus (Group D, n=399), or one of two cyclosporine regimens (Group A, n=390 and Group B, n=399) in combination with MMF and corticosteroids; all patients, except those in one of the two cyclosporine groups, also received induction with daclizumab. The study was conducted outside the United States; the study population was 93% Caucasian. In this study, mortality at 12 months in patients receiving Prograf/MMF was similar (2.7%) compared to patients receiving cyclosporine/MMF (3.3% and 1.8%) or sirolimus/MMF (3.0%). Patients in the Prograf group exhibited higher estimated creatinine clearance rates (eCL_{cr}) using the Cockcroft-Gault formula (Table 1) and experienced fewer efficacy failures, defined as biopsy proven acute rejection (BPAR), graft loss, death, and/or lost to follow-up (Table 2) in comparison to each of the other three groups. Patients randomized to Prograf/MMF were more likely to develop diarrhea and diabetes after the transplantation and experienced similar rates of infections compared to patients randomized to either cyclosporine/MMF regimen (see ADVERSE REACTIONS).

Table 1: Estimated Creatinine Clearance at 12 Months in Study 1

Group	eCLcr [mL/min] at Month 12*				
	N	MEAN	SD	MEDIAN	Treatment Difference with Group C (99.2% CI**)
(A) CsA/MMF/CS	390	56.5	25.8	56.9	-8.6 (-13.7, -3.7)
(B) CsA/MMF/CS/Daclizumab	399	58.9	25.6	60.9	-6.2 (-11.2, -1.2)
(C) Tac/MMF/CS/Daclizumab	401	65.1	27.4	66.2	-
(D) Siro/MMF/CS/Daclizumab	399	56.2	27.4	57.3	-8.9 (-14.1, -3.9)
Total	1589	59.2	26.8	60.5	

Key: CsA=Cyclosporine, CS=Corticosteroids, Tac=Tacrolimus, Siro=Sirolimus

* All death/graft loss (n=41, 27, 23 and 42 in Groups A, B, C and D) and patients whose last recorded creatinine values were prior to month 3 visit (n=10, 9, 7 and 9 in Groups A, B, C and D) were inputed with GFR of 10 mL/min; a subject's last observed creatinine value from month 3 on was used for the remainder of subjects with missing creatinine at month 12 (n=11, 12, 15 and 19 for Groups A, B, C and D). Weight was also imputed in the calculation of estimated GFR, if missing.

** Adjusted for multiple (6) pairwise comparisons using Bonferroni corrections.

Table 2: Incidence of BPAR, Graft Loss, Death or Loss to Follow-up at 12 Months in Study 1

	A N=390	B N=399	C N=401	D N=399
Overall Failure	141 (36.2%)	126 (31.6%)	82 (20.4%)	185 (46.4%)
Components of efficacy failure				
BPAR	113 (29.0%)	106 (26.6%)	60 (15.0%)	152 (38.1%)

Graft loss excluding death	28 (7.2%)	20 (5.0%)	12 (3.0%)	30 (7.5%)
Mortality	13 (3.3%)	7 (1.8%)	11 (2.7%)	12 (3.0%)
Lost to follow-up	5 (1.3%)	7 (1.8%)	5 (1.3%)	6 (1.5%)
Treatment Difference of efficacy failure compared to Group C (99.2% CI*)	15.8% (7.1%, 24.3%)	11.2% (2.7%, 19.5%)	-	26.0% (17.2%, 34.7%)

Group A =CsA/MMF/CS, B =CsA/MMF/CS/Daclizumab, C=Tac/MMF/CS/Daclizumab, and D=Siro/MMF/CS/Daclizumab

* Adjusted for multiple (6) pairwise comparisons using Bonferroni corrections.

The protocol-specified target tacrolimus trough concentrations (C_{trough},Tac) were 3-7 ng/mL; however, the observed median $C_{troughs}$,Tac approximated 7 ng/mL throughout the 12 month study (Table 3).

Table 3: Tacrolimus Whole Blood Trough Concentrations (Study 1)

Time	Median (P10-P90*) tacrolimus whole blood trough concentrations (ng/mL)
Day 30 (N=366)	6.9 (4.4 – 11.3)
Day 90 (N=351)	6.8 (4.1 – 10.7)
Day 180(N=355)	6.5 (4.0 – 9.6)
Day 365 (N=346)	6.5 (3.8 – 10.0)

* Range of C_{trough}, Tac that excludes lowest 10% and highest 10% of C_{trough}, Tac

The protocol-specified target cyclosporine trough concentrations (C_{trough},CsA) for Group B were 50-100 ng/mL; however, the observed median $C_{troughs}$,CsA approximated 100 ng/mL throughout the 12 month study. The protocol-specified target $C_{troughs}$,CsA for Group A were 150-300 ng/mL for the first 3 months and 100-200 ng/mL from month 4 to month 12; the observed median $C_{troughs}$, CsA approximated 225 ng/mL for the first 3 months and 140 ng/mL from month 4 to month 12.

While patients in all groups started MMF at 1g BID, the MMF dose was reduced to <2 g/day in 63% of patients in the tacrolimus treatment arm by month 12 (Table 4); approximately 50% of these MMF dose reductions were due to adverse events. By comparison, the MMF dose was reduced to <2 g/day in 49% and 45% of patients in the two cyclosporine arms (Group A and Group B, respectively), by month 12 and approximately 40% of MMF dose reductions were due to adverse events.

Table 4: MMF Dose Over Time in Prograf/MMF (Group C) (Study 1)

Time period (Days)	Time-averaged MMF dose (g/day)*		
	<2.0	2.0	>2.0
0-30 (N=364)	37%	60%	2%
0-90 (N=373)	47%	51%	2%
0-180 (N=377)	56%	42%	2%
0-365 (N=380)	63%	36%	1%

Time-averaged MMF dose = (total MMF dose)/(duration of treatment)

* Percentage of patients for each time-averaged MMF dose range during various treatment periods. Two g/day of time-averaged MMF dose means that MMF dose was not reduced in those patients during the treatment periods.

In a second randomized, open-label, multi-center trial (Study 2), 424 kidney transplant patients received Prograf (n=212) or cyclosporine (n=212) in combination with MMF 1 gram BID, basiliximab induction, and corticosteroids. In this study, the rate for the combined endpoint of biopsy proven acute rejection, graft failure, death, and/or lost to follow-up at 12 months in the Prograf/MMF group was similar to the rate in the cyclosporine/MMF group. There was, however, an imbalance in mortality at 12 months in those patients receiving Prograf/MMF (4.2%) compared to those receiving cyclosporine/MMF (2.4%), including cases attributed to overimmunosuppression (Table 5).

Table 5: Incidence of BPAR, Graft Loss, Death or Loss to Follow-up at 12 Months in Study 2

	Prograf/MMF (n=212)	Cyclosporine/MMF (n=212)
Overall Failure	32 (15.1%)	36 (17.0%)
Components of efficacy failure		
BPAR	16 (7.5%)	29 (13.7%)
Graft loss excluding death	6 (2.8%)	4 (1.9%)
Mortality	9 (4.2%)	5 (2.4%)
Lost to follow-up	4 (1.9%)	1 (0.5%)
Treatment Difference of efficacy failure compared to Prograf/MMF group (95% CI*)	-	1.9% (-5.2%, 9.0%)

* 95% confidence interval calculated using Fisher's Exact Test

The protocol-specified target tacrolimus whole blood trough concentrations (C_{trough},Tac) in Study 2 were 7-16 ng/mL for the first three months and 5-15 ng/mL thereafter. The observed median $C_{troughs}$,Tac approximated 10 ng/mL during the first three months and 8 ng/mL from month 4 to month 12 (Table 6).

Table 6: Tacrolimus Whole Blood Trough Concentrations (Study 2)

Time	Median (P10-P90*) tacrolimus whole blood trough concentrations (ng/mL)
Day 30 (N=174)	10.5 (6.3 – 16.8)
Day 60 (N=179)	9.2 (5.9 – 15.3)
Day 120 (N=176)	8.3 (4.6 – 13.3)
Day 180 (N=171)	7.8 (5.5 – 13.2)
Day 365 (N=178)	7.1 (4.2 – 12.4)

* Range of C_{trough},Tac that excludes lowest 10% and highest 10% of C_{trough}, Tac

The protocol-specified target cyclosporine whole blood concentrations (C_{trough},CsA) were 125 to 400 ng/mL for the first three months, and 100 to 300 ng/mL thereafter. The observed median $C_{troughs,}$ CsA approximated 280 ng/mL during the first three months and 190 ng/mL from month 4 to month 12.

Patients in both groups started MMF at 1g BID. The MMF dose was reduced to <2 g/day by month 12 in 62% of patients in the Prograf/MMF group (Table 7) and in 47% of patients in the cyclosporine/MMF group. Approximately 63% and 55% of these MMF dose reductions were because of adverse events in the Prograf/MMF group and the cyclosporine/MMF group, respectively.

Table 7: MMF Dose Over Time in the Prograf/MMF group (Study 2)

Time period (Days)	Time-averaged MMF dose (g/day)*		
	<2.0	2.0	>2.0
0-30 (N=212)	25%	69%	6%
0-90 (N=212)	41%	53%	6%
0-180 (N=212)	52%	41%	7%
0-365 (N=212)	62%	34%	4%

Time-averaged MMF dose=(total MMF dose)/(duration of treatment)

* Percentage of patients for each time-averaged MMF dose range during various treatment periods. Two g/day of time-averaged MMF dose means that MMF dose was not reduced in those patients during the treatment periods.

Heart Transplantation

Two open-label, randomized, comparative studies evaluated the safety and efficacy of Prograf-based and cyclosporine-based immunosuppression in primary orthotopic heart transplantation. In a Phase 3 study conducted in Europe, 314 patients received a regimen of antibody induction, corticosteroids and azathioprine in combination with Prograf or cyclosporine modified for 18 months. In a 3-arm study conducted in the US, 331 patients received corticosteroids and Prograf plus sirolimus, Prograf plus mycophenolate mofetil (MMF) or cyclosporine modified plus MMF for 1 year.

In the European Phase 3 study, patient/graft survival at 18 months posttransplant was similar between treatment arms, 91.7% in the tacrolimus group and 89.2% in the cyclosporine group. In the US study, patient and graft survival at 12 months was similar with 93.5% survival in the Prograf plus MMF group and 86.1% survival in the cyclosporine modified plus MMF group. In the European study, the cyclosporine trough concentrations were above the pre-defined target range (i.e., 100-200 ng/mL) at Day 122 and beyond in 32-68% of the patients in the cyclosporine treatment arm, whereas the tacrolimus trough concentrations were within the pre-defined target range (i.e., 5-15 ng/mL) in 74-86% of the patients in the tacrolimus treatment arm.

The US study contained a third arm of a combination regimen of sirolimus, 2 mg per day, and full-dose Prograf; however, this regimen was associated with increased risk of wound healing complications, renal function impairment, and insulin-dependent post-transplant diabetes mellitus, and is not recommended (see WARNINGS).

INDICATIONS AND USAGE

Prograf is indicated for the prophylaxis of organ rejection in patients receiving allogeneic liver, kidney, or heart transplants. It is recommended that Prograf be used concomitantly with adrenal corticosteroids. Because of the risk of anaphylaxis, Prograf injection should be reserved for patients unable to take Prograf capsules orally. In heart and kidney transplant recipients, it is recommended that Prograf be used in conjunction with azathioprine or mycophenolate mofetil (MMF). The safety and efficacy of the use of Prograf with sirolimus has not been established (see CLINICAL STUDIES).

CONTRAINDICATIONS

Prograf is contraindicated in patients with a hypersensitivity to tacrolimus. Prograf injection is contraindicated in patients with a hypersensitivity to HCO-60 (polyoxyl 60 hydrogenated castor oil).

WARNINGS

(See boxed WARNING)

Post-Transplant Diabetes Mellitus

Insulin-dependent post-transplant diabetes mellitus (PTDM) was reported in 20% of Prograf-treated kidney transplant patients without pretransplant history of diabetes mellitus in the Phase III study (See Tables Below). The median time to onset of PTDM was 68 days. Insulin dependence was reversible in 15% of these PTDM patients at one year and in 50% at 2 years post transplant. Black and Hispanic kidney transplant patients were at an increased risk of development of PTDM.

Incidence of Post Transplant Diabetes Mellitus and Insulin Use at 2 Years in Kidney Transplant Recipients in the Phase III study

Status of PTDM*	Prograf	CBIR
Patients without pretransplant history of diabetes mellitus.	151	151
New onset PTDM*, 1st Year	30/151 (20%)	6/151 (4%)
Still insulin dependent at one year in those without prior history of diabetes.	25/151 (17%)	5/151 (3%)
New onset PTDM* post 1 year	1	0
Patients with PTDM* at 2 years	16/151 (11%)	5/151 (3%)

* use of insulin for 30 or more consecutive days, with < 5 day gap, without a prior history of insulin dependent diabetes mellitus or non insulin dependent diabetes mellitus.

Development of Post Transplant Diabetes Mellitus by Race and by Treatment Group during First Year Post Kidney Transplantation in the Phase III study

		Prograf		CBIR
Patient Race	**No. of Patients at Risk**	**Patients Who Developed PTDM***	**No. of Patients At Risk**	**Patients Who Developed PTDM***
Black	41	15 (37%)	36	3 (8%)
Hispanic	17	5 (29%)	18	1 (6%)

Caucasian	82	10 (12%)	87	1 (1%)
Other	11	0 (0%)	10	1 (10%)
Total	151	30 (20%)	151	6 (4%)

* use of insulin for 30 or more consecutive days, with < 5 day gap, without a prior history of insulin dependent diabetes mellitus or non insulin dependent diabetes mellitus.

Insulin-dependent post-transplant diabetes mellitus was reported in 18% and 11% of Prograf-treated liver transplant patients and was reversible in 45% and 31% of these patients at 1 year post transplant, in the U.S. and European randomized studies, respectively (See Table below). Hyperglycemia was associated with the use of Prograf in 47% and 33% of liver transplant recipients in the U.S. and European randomized studies, respectively, and may require treatment (see ADVERSE REACTIONS).

Incidence of Post Transplant Diabetes Mellitus and Insulin Use at 1 Year in Liver Transplant Recipients

Status of PTDM*	**US Study**		**European Study**	
	Prograf	**CBIR**	**Prograf**	**CBIR**
Patients at risk**	239	236	239	249
New Onset PTDM*	42 (18%)	30 (13%)	26 (11%)	12 (5%)
Patients still on insulin at 1 year	23 (10%)	19 (8%)	18 (8%)	6 (2%)

* use of insulin for 30 or more consecutive days, with < 5 day gap, without a prior history of insulin dependent diabetes mellitus or non insulin dependent diabetes mellitus.

** Patients without pretransplant history of diabetes mellitus.

Insulin-dependent post-transplant diabetes mellitus was reported in 13% and 22% of Prograf-treated heart transplant patients receiving mycophenolate mofetil or azathioprine and was reversible in 30% and 17% of these patients at one year post transplant, in the US and European randomized studies, respectively (See Table below). Hyperglycemia defined as two fasting plasma glucose levels ≥126 mg/dL was reported with the use of Prograf plus mycophenolate mofetil or azathioprine in 32% and 35% of heart transplant recipients in the US and European randomized studies, respectively, and may require treatment (see ADVERSE REACTIONS).

Incidence of Post Transplant Diabetes Mellitus and Insulin Use at 1 Year in Heart Transplant Recipients

Status of PTDM*	US Study			European Study	
	Prograf/Sirolimus	Prograf/ MMF	Cyclosporine/ MMF	Prograf/ AZA	Cyclosporine/ AZA
Patients at risk**	85	75	83	132	138
New Onset PTDM*	21 (25%)	10 (13%)	6 (7%)	29 (22%)	5 (4%)
Patients still on insulin at 1 year***	10 (12%)	7 (9%)	1 (1%)	24 (18%)	4 (3%)

* use of insulin for 30 or more consecutive days without a prior history of insulin dependent diabetes mellitus or non insulin dependent diabetes mellitus.

** Patients without pretransplant history of diabetes mellitus.

*** 7-12 months for the US Study.

Nephrotoxicity

Prograf can cause nephrotoxicity, particularly when used in high doses. Nephrotoxicity was reported in approximately 52% of kidney transplantation patients and in 40% and 36% of liver transplantation patients receiving Prograf in the U.S. and European randomized trials, respectively, and in 59% of heart transplantation patients in a European randomized trial (see ADVERSE REACTIONS). Use of Prograf with sirolimus in heart transplantation patients in a US study was associated with increased risk of renal function impairment, and is not recommended (See CLINICAL STUDIES). More overt nephrotoxicity is seen early after transplantation, characterized by increasing serum creatinine and a decrease in urine output. Patients with impaired renal function should be monitored closely as the dosage of Prograf may need to be reduced. In patients with persistent elevations of serum creatinine who are unresponsive to dosage adjustments, consideration should be given to changing to another immunosuppressive therapy. Care should be taken in using tacrolimus with other nephrotoxic drugs. **In particular, to avoid excess nephrotoxicity, Prograf should not be used simultaneously with cyclosporine. Prograf or cyclosporine should be discontinued at least 24 hours prior to initiating the other. In the presence of elevated Prograf or cyclosporine concentrations, dosing with the other drug usually should be further delayed.**

Hyperkalemia

Mild to severe hyperkalemia was reported in 31% of kidney transplant recipients and in 45% and 13% of liver transplant recipients treated with Prograf in the U.S. and European randomized trials, respectively,

and in 8% of heart transplant recipients in a European randomized trial and may require treatment (see ADVERSE REACTIONS). **Serum potassium levels should be monitored and potassium-sparing diuretics should not be used during Prograf therapy** (see PRECAUTIONS).

Neurotoxicity

Prograf can cause neurotoxicity, particularly when used in high doses. Neurotoxicity, including tremor, headache, and other changes in motor function, mental status, and sensory function were reported in approximately 55% of liver transplant recipients in the two randomized studies. Tremor occurred more often in Prograf-treated kidney transplant patients (54%) and heart transplant patients (15%) compared to cyclosporine-treated patients. The incidence of other neurological events in kidney transplant and heart transplant patients was similar in the two treatment groups (see ADVERSE REACTIONS). Tremor and headache have been associated with high whole-blood concentrations of tacrolimus and may respond to dosage adjustment. Seizures have occurred in adult and pediatric patients receiving Prograf (see ADVERSE REACTIONS). Coma and delirium also have been associated with high plasma concentrations of tacrolimus.

Patients treated with tacrolimus have been reported to develop posterior reversible encephalopathy syndrome (PRES). Symptoms indicating PRES include headache, altered mental status, seizures, visual disturbances and hypertension. Diagnosis may be confirmed by radiological procedure. If PRES is suspected or diagnosed, blood pressure control should be maintained and immediate reduction of immunosuppression is advised. This syndrome is characterized by reversal of symptoms upon reduction or discontinuation of immunosuppression.

Malignancy and Lymphoproliferative Disorders

As in patients receiving other immunosuppressants, patients receiving Prograf are at increased risk of developing lymphomas and other malignancies, particularly of the skin. The risk appears to be related to the intensity and duration of immunosuppression rather than to the use of any specific agent. A lymphoproliferative disorder (LPD) related to Epstein-Barr Virus (EBV) infection has been reported in immunosuppressed organ transplant recipients. The risk of LPD appears greatest in young children who are at risk for primary EBV infection while immunosuppressed or who are switched to Prograf following long-term immunosuppression therapy. Because of the danger of oversuppression of the immune system which can increase susceptibility to infection, combination immunosuppressant therapy should be used with caution.

Latent Viral Infections

Immunosuppressed patients are at increased risk for opportunistic infections, including activation of latent viral infections. These include BK virus associated nephropathy and JC virus associated progressive multifocal leukoencephalopathy (PML) which have been observed in patients receiving tacrolimus. These infections may lead to serious, including fatal, outcomes.

Prograf in Combination with Sirolimus

The use of full-dose Prograf with sirolimus (2 mg per day) in heart transplant recipients was associated with increased risk of wound healing complications, renal function impairment, and insulin-dependent post-transplant diabetes mellitus, and is not recommended (see CLINICAL STUDIES).

Anaphylactic Reactions

A few patients receiving Prograf injection have experienced anaphylactic reactions. Although the exact cause of these reactions is not known, other drugs with castor oil derivatives in the formulation have been associated with anaphylaxis in a small percentage of patients. Because of this potential risk of anaphylaxis, Prograf injection should be reserved for patients who are unable to take Prograf capsules.

Patients receiving Prograf injection should be under continuous observation for at least the first 30 minutes following the start of the infusion and at frequent intervals thereafter. If signs or symptoms of anaphylaxis occur, the infusion should be stopped. An aqueous solution of epinephrine should be available at the bedside as well as a source of oxygen.

PRECAUTIONS

General

Hypertension is a common adverse effect of Prograf therapy (see ADVERSE REACTIONS). Mild or moderate hypertension is more frequently reported than severe hypertension. Antihypertensive therapy may be required; the control of blood pressure can be accomplished with any of the common antihypertensive agents. Since tacrolimus may cause hyperkalemia, potassium-sparing diuretics should be avoided. While calcium-channel blocking agents can be effective in treating Prograf-associated hypertension, care should be taken since interference with tacrolimus metabolism may require a dosage reduction (see Drug Interactions).

Renally and Hepatically Impaired Patients

For patients with renal insufficiency some evidence suggests that lower doses should be used (see CLINICAL PHARMACOLOGY and DOSAGE AND ADMINISTRATION).

The use of Prograf in liver transplant recipients experiencing post-transplant hepatic impairment may be associated with increased risk of developing renal insufficiency related to high whole-blood levels of tacrolimus. These patients should be monitored closely and dosage adjustments should be considered. Some evidence suggests that lower doses should be used in these patients (see DOSAGE AND ADMINISTRATION).

Myocardial Hypertrophy

Myocardial hypertrophy has been reported in association with the administration of Prograf, and is generally manifested by echocardiographically demonstrated concentric increases in left ventricular posterior wall and interventricular septum thickness. Hypertrophy has been observed in infants, children and adults. This condition appears reversible in most cases following dose reduction or discontinuance of therapy. In a group of 20 patients with pre- and post-treatment echocardiograms who showed evidence of myocardial hypertrophy, mean tacrolimus whole blood concentrations during the period prior to diagnosis of myocardial hypertrophy ranged from 11 to 53 ng/mL in infants (N=10, age 0.4 to 2 years), 4 to 46 ng/mL in children (N=7, age 2 to 15 years) and 11 to 24 ng/mL in adults (N=3, age 37 to 53 years).

In patients who develop renal failure or clinical manifestations of ventricular dysfunction while receiving Prograf therapy, echocardiographic evaluation should be considered. If myocardial hypertrophy is diagnosed, dosage reduction or discontinuation of Prograf should be considered.

Information for Patients

Patients should be informed of the need for repeated appropriate laboratory tests while they are receiving Prograf. They should be given complete dosage instructions, advised of the potential risks during pregnancy, and informed of the increased risk of neoplasia. Patients should be informed that changes in dosage should not be undertaken without first consulting their physician.

Patients should be informed that Prograf can cause diabetes mellitus and should be advised of the need to see their physician if they develop frequent urination, increased thirst or hunger.

As with other immunosuppressive agents, owing to the potential risk of malignant skin changes, exposure to sunlight and ultraviolet (UV) light should be limited by wearing protective clothing and using a sunscreen with a high protection factor.

Laboratory Tests

Serum creatinine, potassium, and fasting glucose should be assessed regularly. Routine monitoring of metabolic and hematologic systems should be performed as clinically warranted.

Drug Interactions

Due to the potential for additive or synergistic impairment of renal function, care should be taken when administering Prograf with drugs that may be associated with renal dysfunction. These include, but are not limited to, aminoglycosides, amphotericin B, and cisplatin. Initial clinical experience with the co-administration of Prograf and cyclosporine resulted in additive/synergistic nephrotoxicity. Patients switched from cyclosporine to Prograf should receive the first Prograf dose no sooner than 24 hours after the last cyclosporine dose. Dosing may be further delayed in the presence of elevated cyclosporine levels.

Drugs that May Alter Tacrolimus Concentrations

Since tacrolimus is metabolized mainly by the CYP3A enzyme systems, substances known to inhibit these enzymes may decrease the metabolism or increase bioavailability of tacrolimus as indicated by increased whole blood or plasma concentrations. Drugs known to induce these enzyme systems may result in an increased metabolism of tacrolimus or decreased bioavailability as indicated by decreased whole blood or plasma concentrations. Monitoring of blood concentrations and appropriate dosage adjustments are essential when such drugs are used concomitantly.

Drugs That May Increase Tacrolimus Blood Concentrations

Calcium Channel Blockers	**Antifungal Agents**	**Macrolide Antibiotics**
diltiazem	clotrimazole	clarithromycin
nicardipine	fluconazole	erythromycin
nifedipine	itraconazole	troleandomycin
verapamil	ketoconazole**	
	voriconazole	
Gastrointestinal Prokinetic Agents	**Other Drugs**	
cisapride	bromocriptine	

metoclopramide	chloramphenicol cimetidine cyclosporine danazol ethinyl estradiol methylprednisolone lansoprazole*** omeprazole protease inhibitors nefazodone magnesium-aluminum-hydroxide

* This table is not all inclusive.

** In a study of 6 normal volunteers, a significant increase in tacrolimus oral bioavailability (14±5% vs. 30±8%) was observed with concomitant ketoconazole administration (200 mg). The apparent oral clearance of tacrolimus during ketoconazole administration was significantly decreased compared to tacrolimus alone (0.430±0.129 L/hr/kg vs. 0.148±0.043 L/hr/kg). Overall, IV clearance of tacrolimus was not significantly changed by ketoconazole co-administration, although it was highly variable between patients.

*** Lansoprazole (CYP2C19, CYP3A4 substrate) may potentially inhibit CYP3A4-mediated metabolism of tacrolimus and thereby substantially increase tacrolimus whole blood concentrations, especially in transplant patients who are intermediate or poor CYP2C19 metabolizers, as compared to those patients who are efficient CYP2C19 metabolizers.

****Drugs That May Decrease Tacrolimus Blood Concentrations***

Anticonvulsants	**Antimicrobials**
carbamazepine	rifabutin
phenobarbital	caspofungin
phenytoin	rifampin
Herbal Preparations	**Other Drugs**
St. John's Wort	sirolimus

* This table is not all inclusive.

St. John's Wort (*Hypericum perforatum)* induces CYP3A4 and P-glycoprotein. Since tacrolimus is a substrate for CYP3A4, there is the potential that the use of St. John's Wort in patients receiving Prograf could result in reduced tacrolimus levels.

In a single-dose crossover study in healthy volunteers, co-administration of tacrolimus and magnesium-aluminum-hydroxide resulted in a 21% increase in the mean tacrolimus AUC and a 10% decrease in the mean tacrolimus C_{max} relative to tacrolimus administration alone.

In a study of 6 normal volunteers, a significant decrease in tacrolimus oral bioavailability (14±6% vs. 7±3%) was observed with concomitant rifampin administration (600 mg). In addition, there was a significant increase in tacrolimus clearance (0.036±0.008 L/hr/kg vs. 0.053±0.010 L/hr/kg) with concomitant rifampin administration.

Interaction studies with drugs used in HIV therapy have not been conducted. However, care should be exercised when drugs that are nephrotoxic (e.g., ganciclovir) or that are metabolized by CYP3A (e.g., nelfinavir, ritonavir) are administered concomitantly with tacrolimus. Based on a clinical study of 5 liver transplant recipients, co-administration of tacrolimus with nelfinavir increased blood concentrations of tacrolimus significantly and, as a result, a reduction in the tacrolimus dose by an average of 16-fold was

needed to maintain mean trough tacrolimus blood concentrations of 9.7 ng/mL. Thus, frequent monitoring of tacrolimus blood concentrations and appropriate dosage adjustments are essential when nelfinavir is used concomitantly. Tacrolimus may affect the pharmacokinetics of other drugs (e.g., phenytoin) and increase their concentration. Grapefruit juice affects CYP3A-mediated metabolism and should be avoided (see DOSAGE AND ADMINISTRATION).

Following co-administration of tacrolimus and sirolimus (2 or 5 mg/day) in stable renal transplant patients, mean tacrolimus AUC_{0-12} and C_{min} decreased approximately by 30% relative to tacrolimus alone. Mean tacrolimus AUC_{0-12} and C_{min} following co-administration of 1 mg/day of sirolimus decreased approximately 3% and 11%, respectively. The safety and efficacy of tacrolimus used in combination with sirolimus for the prevention of graft rejection has not been established and is not recommended.

Other Drug Interactions

Immunosuppressants may affect vaccination. Therefore, during treatment with Prograf, vaccination may be less effective. The use of live vaccines should be avoided; live vaccines may include, but are not limited to measles, mumps, rubella, oral polio, BCG, yellow fever, and TY 21a typhoid.[1]

At a given MMF dose, mycophenolic acid (MPA) exposure is higher with Prograf co-administration than with cyclosporine co-administration due to the differences in the interruption of the enterohepatic recirculation of MPA. Clinicians should be aware that there is also a potential for increased MPA exposure after crossover from cyclosporine to tacrolimus in patients concomitantly receiving MMF or MPA.

Carcinogenesis, Mutagenesis and Impairment of Fertility

An increased incidence of malignancy is a recognized complication of immunosuppression in recipients of organ transplants. The most common forms of neoplasms are non-Hodgkin's lymphomas and carcinomas of the skin. As with other immunosuppressive therapies, the risk of malignancies in Prograf recipients may be higher than in the normal, healthy population. Lymphoproliferative disorders associated with Epstein-Barr Virus infection have been seen. It has been reported that reduction or discontinuation of immunosuppression may cause the lesions to regress.

No evidence of genotoxicity was seen in bacterial (*Salmonella* and *E. coli*) or mammalian (Chinese hamster lung-derived cells) in vitro assays of mutagenicity, the in vitro CHO/HGPRT assay of mutagenicity, or in vivo clastogenicity assays performed in mice; tacrolimus did not cause unscheduled DNA synthesis in rodent hepatocytes.

Carcinogenicity studies were carried out in male and female rats and mice. In the 80-week mouse study and in the 104-week rat study no relationship of tumor incidence to tacrolimus dosage was found. The highest doses used in the mouse and rat studies were 0.8 – 2.5 times (mice) and 3.5 – 7.1 times (rats) the recommended clinical dose range of 0.1 – 0.2 mg/kg/day when corrected for body surface area.

No impairment of fertility was demonstrated in studies of male and female rats. Tacrolimus, given orally at 1.0 mg/kg (0.7 – 1.4X the recommended clinical dose range of 0.1 – 0.2 mg/kg/day based on body surface area corrections) to male and female rats, prior to and during mating, as well as to dams during gestation and lactation, was associated with embryolethality and with adverse effects on female reproduction. Effects on female reproductive function (parturition) and embryolethal effects were indicated by a higher rate of pre-implantation loss and increased numbers of undelivered and nonviable pups. When given at 3.2 mg/kg (2.3 – 4.6X the recommended clinical dose range based on body surface area correction), tacrolimus was associated with maternal and paternal toxicity as well as reproductive toxicity including marked adverse effects on estrus cycles, parturition, pup viability, and pup malformations.

Pregnancy: Category C

In reproduction studies in rats and rabbits, adverse effects on the fetus were observed mainly at dose levels that were toxic to dams. Tacrolimus at oral doses of 0.32 and 1.0 mg/kg during organogenesis in rabbits was associated with maternal toxicity as well as an increase in incidence of abortions; these doses are equivalent to 0.5 – 1X and 1.6 – 3.3X the recommended clinical dose range (0.1 – 0.2 mg/kg) based on body surface area corrections. At the higher dose only, an increased incidence of malformations and developmental variations was also seen. Tacrolimus, at oral doses of 3.2 mg/kg during organogenesis in rats, was associated with maternal toxicity and caused an increase in late resorptions, decreased numbers of live births, and decreased pup weight and viability. Tacrolimus, given orally at 1.0 and 3.2 mg/kg (equivalent to 0.7 – 1.4X and 2.3 – 4.6X the recommended clinical dose range based on body surface area corrections) to pregnant rats after organogenesis and during lactation, was associated with reduced pup weights.

No reduction in male or female fertility was evident.

There are no adequate and well-controlled studies in pregnant women. Tacrolimus is transferred across the placenta. The use of tacrolimus during pregnancy has been associated with neonatal hyperkalemia and renal dysfunction. Prograf should be used during pregnancy only if the potential benefit to the mother justifies potential risk to the fetus.

Nursing Mothers

Since tacrolimus is excreted in human milk, nursing should be avoided.

Pediatric Patients

Experience with Prograf in pediatric kidney and heart transplant patients is limited. Successful liver transplants have been performed in pediatric patients (ages up to 16 years) using Prograf. Two randomized active-controlled trials of Prograf in primary liver transplantation included 56 pediatric patients. Thirty-one patients were randomized to Prograf-based and 25 to cyclosporine-based therapies. Additionally, a minimum of 122 pediatric patients were studied in an uncontrolled trial of tacrolimus in living related donor liver transplantation. Pediatric patients generally required higher doses of Prograf to maintain blood trough concentrations of tacrolimus similar to adult patients (see DOSAGE AND ADMINISTRATION).

ADVERSE REACTIONS

Liver Transplantation

The principal adverse reactions of Prograf are tremor, headache, diarrhea, hypertension, nausea, and abnormal renal function. These occur with oral and IV administration of Prograf and may respond to a reduction in dosing. Diarrhea was sometimes associated with other gastrointestinal complaints such as nausea and vomiting.

Hyperkalemia and hypomagnesemia have occurred in patients receiving Prograf therapy. Hyperglycemia has been noted in many patients; some may require insulin therapy (see WARNINGS).

The incidence of adverse events was determined in two randomized comparative liver transplant trials among 514 patients receiving tacrolimus and steroids and 515 patients receiving a cyclosporine-based regimen (CBIR). The proportion of patients reporting more than one adverse event was 99.8% in the tacrolimus group and 99.6% in the CBIR group. Precautions must be taken when comparing the incidence

of adverse events in the U.S. study to that in the European study. The 12-month posttransplant information from the U.S. study and from the European study is presented below. The two studies also included different patient populations and patients were treated with immunosuppressive regimens of differing intensities. Adverse events reported in ≥ 15% in tacrolimus patients (combined study results) are presented below for the two controlled trials in liver transplantation:

LIVER TRANSPLANTATION: ADVERSE EVENTS OCCURRING IN ≥ 15% OF PROGRAF-TREATED PATIENTS

	U.S. STUDY		EUROPEAN STUDY	
	Prograf (N=250)	CBIR (N=250)	Prograf (N=264)	CBIR (N=265)
Nervous System				
Headache (see WARNINGS)	64%	60%	37%	26%
Tremor (see WARNINGS)	56%	46%	48%	32%
Insomnia	64%	68%	32%	23%
Paresthesia	40%	30%	17%	17%
Gastrointestinal				
Diarrhea	72%	47%	37%	27%
Nausea	46%	37%	32%	27%
Constipation	24%	27%	23%	21%
LFT Abnormal	36%	30%	6%	5%
Anorexia	34%	24%	7%	5%
Vomiting	27%	15%	14%	11%
Cardiovascular				
Hypertension (see PRECAUTIONS)	47%	56%	38%	43%
Urogenital				
Kidney Function Abnormal (see WARNINGS)	40%	27%	36%	23%
Creatinine Increased (see WARNINGS)	39%	25%	24%	19%
BUN Increased (see WARNINGS)	30%	22%	12%	9%
Urinary Tract Infection	16%	18%	21%	19%
Oliguria	18%	15%	19%	12%
Metabolic and Nutritional				

Hyperkalemia (see WARNINGS)	45%	26%	13%	9%
Hypokalemia	29%	34%	13%	16%
Hyperglycemia (see WARNINGS)	47%	38%	33%	22%
Hypomagnesemia	48%	45%	16%	9%
Hemic and Lymphatic				
Anemia	47%	38%	5%	1%
Leukocytosis	32%	26%	8%	8%
Thrombocytopenia	24%	20%	14%	19%
Miscellaneous				
Abdominal Pain	59%	54%	29%	22%
Pain	63%	57%	24%	22%
Fever	48%	56%	19%	22%
Asthenia	52%	48%	11%	7%
Back Pain	30%	29%	17%	17%
Ascites	27%	22%	7%	8%
Peripheral Edema	26%	26%	12%	14%
Respiratory System				
Pleural Effusion	30%	32%	36%	35%
Atelectasis	28%	30%	5%	4%
Dyspnea	29%	23%	5%	4%
Skin and Appendages				
Pruritus	36%	20%	15%	7%
Rash	24%	19%	10%	4%

Less frequently observed adverse reactions in both liver transplantation and kidney transplantation patients are described under the subsection Less Frequently Reported Adverse Reactions below.

Kidney Transplantation

The most common adverse reactions reported were infection, tremor, hypertension, abnormal renal function, constipation, diarrhea, headache, abdominal pain and insomnia.

Adverse events that occurred in ≥ 15% of kidney transplant patients treated with Prograf in conjunction with azathioprine are presented below:

KIDNEY TRANSPLANTATION: ADVERSE EVENTS OCCURRING IN ≥ 15% OF PATIENTS TREATED WITH PROGRAF IN CONJUNCTION WITH AZATHIOPRINE

	Prograf (N=205)	CBIR (N=207)
Nervous System		
Tremor (see WARNINGS)	54%	34%
Headache (see WARNINGS)	44%	38%
Insomnia	32%	30%
Paresthesia	23%	16%
Dizziness	19%	16%
Gastrointestinal		
Diarrhea	44%	41%
Nausea	38%	36%
Constipation	35%	43%
Vomiting	29%	23%
Dyspepsia	28%	20%
Cardiovascular		
Hypertension (see PRECAUTIONS)	50%	52%
Chest pain	19%	13%
Urogenital		
Creatinine Increased (see WARNINGS)	45%	42%
Urinary Tract Infection	34%	35%
Metabolic and Nutritional		
Hypophosphatemia	49%	53%
Hypomagnesemia	34%	17%
Hyperlipemia	31%	38%
Hyperkalemia (see WARNINGS)	31%	32%
Diabetes Mellitus (see WARNINGS)	24%	9%
Hypokalemia	22%	25%
Hyperglycemia (see WARNINGS)	22%	16%
Edema	18%	19%

Hemic and Lymphatic		
Anemia	30%	24%
Leukopenia	15%	17%
Miscellaneous		
Infection	45%	49%
Peripheral Edema	36%	48%
Asthenia	34%	30%
Abdominal Pain	33%	31%
Pain	32%	30%
Fever	29%	29%
Back Pain	24%	20%
Respiratory System		
Dyspnea	22%	18%
Cough Increased	18%	15%
Musculoskeletal		
Arthralgia	25%	24%
Skin		
Rash	17%	12%
Pruritus	15%	7%

Adverse events that occurred in ≥ 10% of kidney transplant patients treated with Prograf in conjunction with MMF in Study 1* are presented below:

KIDNEY TRANSPLANTATION: ADVERSE EVENTS OCCURRING IN ≥ 10% OF PROGRAF-TREATED PATIENTS			
	Prograf (Group C) (N=403)	**Cyclosporine (Group A) (N=384)**	**Cyclosporine (Group B) (N=408)**
Anemia	17%	19%	17%
Leucopenia	13%	10%	10%
Diarrhea	25%	16%	13%
Edema peripheral	11%	12%	13%
Urinary tract infection	24%	28%	24%
Hyperlipidemia	10%	15%	13%
Hypertension (see PRECAUTIONS)	13%	14%	12%

*Study 1 was conducted entirely outside of the United States. Such studies often report a lower incidence of adverse events in comparison to US studies.

Adverse events that occurred in ≥15% of kidney transplant patients treated with Prograf in conjunction with MMF in Study 2 are presented below:

KIDNEY TRANSPLANTATION: ADVERSE EVENTS OCCURRING IN ≥ 15% OF PROGRAF-TREATED PATIENTS		
	Prograf (N=212)	**Cyclosporine (N=212)**
Gastrointestinal Disorders		
Diarrhea	44%	26%
Nausea	39%	47%
Constipation	36%	41%
Vomiting	26%	25%
Dyspepsia	18%	15%
Injury, Poisoning, and Procedural Complications		
Post Procedural Pain	29%	27%
Incision Site Complication	28%	23%
Graft Dysfunction	24%	18%
Metabolism and Nutrition Disorders		
Hypomagnesemia	28%	22%
Hypophosphatemia	28%	21%
Hyperkalemia (see WARNINGS)	26%	19%
Hyperglycemia (see WARNINGS)	21%	15%
Hyperlipidemia	18%	25%
Hypokalemia	16%	18%
Nervous System Disorders		
Tremor	34%	20%
Headache	24%	25%
Blood and Lymphatic System Disorders		
Anemia	30%	28%
Leukopenia	16%	12%
Miscellaneous		
Edema Peripheral	35%	46%
Hypertension (see PRECAUTIONS)	32%	35%
Insomnia	30%	21%
Urinary Tract Infection	26%	22%
Blood creatinine increased	23%	23%

Less frequently observed adverse reactions in both liver transplantation and kidney transplantation patients are described under the subsection Less Frequently Reported Adverse Reactions shown below.

Heart Transplantation

The more common adverse reactions in Prograf-treated heart transplant recipients were abnormal renal function , hypertension, diabetes mellitus, CMV infection, tremor, hyperglycemia, leukopenia, infection, and hyperlipemia.

Adverse events in heart transplant patients in the European trial are presented below:

HEART TRANSPLANTATION: ADVERSE EVENTS OCCURRING IN ≥ 15% OF PROGRAF-TREATED PATIENTS

COSTART Body System COSTART Term	Prograf+ Azathioprine (n=157)	CsA + Azathioprine (n=157)
Cardiovascular System		
Hypertension (See PRECAUTIONS)	62%	69%
Pericardial effusion	15%	14%
Body as a Whole		
CMV infection	32%	30%
Infection	24%	21%
Metabolic and Nutritional Disorders		
Hyperlipemia	18%	27%
Diabetes Mellitus (See WARNINGS)	26%	16%
Hyperglycemia (See WARNINGS)	23%	17%
Hemic and Lymphatic System		
Leukopenia	48%	39%
Anemia	50%	36%
Urogenital System		
Kidney function abnormal (See WARNINGS)	56%	57%
Urinary tract infection	16%	12%
Respiratory System		
Bronchitis	17%	18%
Nervous System		
Tremor (See WARNINGS)	15%	6%

In the European study, the cyclosporine trough concentrations were above the pre-defined target range (i.e., 100-200 ng/mL) at Day 122 and beyond in 32-68% of the patients in the cyclosporine treatment arm, whereas the tacrolimus trough concentrations were within the pre-defined target range (i.e., 5-15 ng/mL) in 74-86% of the patients in the tacrolimus treatment arm.

Only selected targeted treatment-emergent adverse events were collected in the US heart transplantation study. Those events that were reported at a rate of 15% or greater in patients treated with Prograf and mycophenolate mofetil include the following: any target adverse events (99.1%), hypertension (88.8%), hyperglycemia requiring antihyperglycemic therapy (70.1%) (see WARNINGS), hypertriglyceridemia (65.4%), anemia (hemoglobin <10.0 g/dL) (65.4%), fasting blood glucose >140 mg/dL (on two separate occasions) (60.7%) (see WARNINGS), hypercholesterolemia (57.0%), hyperlipidemia (33.6%), WBCs <3000 cells/mcL (33.6%), serious bacterial infections (29.9%), magnesium <1.2 mEq/L (24.3%), platelet count <75,000 cells/mcL (18.7%), and other opportunistic infections (15.0%).

Other targeted treatment-emergent adverse events in Prograf-treated patients occurred at a rate of less than 15%, and include the following: Cushingoid features, impaired wound healing, hyperkalemia, *Candida* infection, and CMV infection/syndrome.

Less Frequently Reported Adverse Reactions

The following adverse events were reported in either liver, kidney, and/or heart transplant recipients who were treated with tacrolimus in clinical trials.

Nervous System *(see* WARNINGS*)*
Abnormal dreams, agitation, amnesia, anxiety, confusion, convulsion, crying, depression, dizziness, elevated mood, emotional lability, encephalopathy, haemorrhagic stroke, hallucinations, headache, hypertonia, incoordination, insomnia, monoparesis, myoclonus, nerve compression, nervousness, neuralgia, neuropathy, paresthesia, paralysis flaccid, psychomotor skills impaired, psychosis, quadriparesis, somnolence, thinking abnormal, vertigo, writing impaired

Special Senses
Abnormal vision, amblyopia, ear pain, otitis media, tinnitus

Gastrointestinal
Anorexia, cholangitis, cholestatic jaundice, diarrhea, duodenitis, dyspepsia, dysphagia, esophagitis, flatulence, gastritis, gastroesophagitis, gastrointestinal hemorrhage, GGT increase, GI disorder, GI perforation, hepatitis, hepatitis granulomatous, ileus, increased appetite, jaundice, liver damage, liver function test abnormal, nausea, nausea and vomiting, oesophagitis ulcerative, oral moniliasis, pancreatic pseudocyst, rectal disorder, stomatitis, vomiting

Cardiovascular
Abnormal ECG, angina pectoris, arrhythmia, atrial fibrillation, atrial flutter, bradycardia, cardiac fibrillation, cardiopulmonary failure, cardiovascular disorder, chest pain, congestive heart failure, deep thrombophlebitis, echocardiogram abnormal, electrocardiogram QRS complex abnormal, electrocardiogram ST segment abnormal, heart failure, heart rate decreased, hemorrhage, hypotension, peripheral vascular disorder, phlebitis, postural hypotension, syncope, tachycardia, thrombosis, vasodilatation

Urogenital *(see* WARNINGS*)*
Acute kidney failure, albuminuria, BK nephropathy, bladder spasm, cystitis, dysuria, hematuria, hydronephrosis, kidney failure, kidney tubular necrosis, nocturia, oliguria, pyuria, toxic nephropathy, urge incontinence, urinary frequency, urinary incontinence, urinary retention, vaginitis

Metabolic/Nutritional
Acidosis, alkaline phosphatase increased, alkalosis, ALT (SGPT) increased, AST (SGOT) increased, bicarbonate decreased, bilirubinemia, BUN increased, dehydration, edema, GGT increased, gout, healing abnormal, hypercalcemia, hypercholesterolemia, hyperkalemia, hyperlipemia, hyperphosphatemia, hyperuricemia, hypervolemia, hypocalcemia, hypoglycemia, hypokalemia, hypomagnesemia, hyponatremia, hypophosphatemia, hypoproteinemia, lactic dehydrogenase increase, peripheral edema, weight gain

Endocrine *(see* PRECAUTIONS*)*

Cushing's syndrome, diabetes mellitus

Hemic/Lymphatic

Coagulation disorder, ecchymosis, haematocrit increased, haemoglobin abnormal, hypochromic anemia, leukocytosis, leukopenia, polycythemia, prothrombin decreased, serum iron decreased, thrombocytopenia

Miscellaneous

Abdomen enlarged, abdominal pain, abscess, accidental injury, allergic reaction, asthenia, back pain, cellulitis, chills, fall, feeling abnormal, fever, flu syndrome, generalized edema, hernia, mobility decreased, pain, peritonitis, photosensitivity reaction, sepsis, temperature intolerance, ulcer

Musculoskeletal

Arthralgia, cramps, generalized spasm, joint disorder, leg cramps, myalgia, myasthenia, osteoporosis

Respiratory

Asthma, bronchitis, cough increased, dyspnea, emphysema, hiccups, lung disorder, lung function decreased, pharyngitis, pleural effusion, pneumonia, pneumothorax, pulmonary edema, respiratory disorder, rhinitis, sinusitis, voice alteration

Skin

Acne, alopecia, exfoliative dermatitis, fungal dermatitis, herpes simplex, herpes zoster, hirsutism, neoplasm skin benign, skin discoloration, skin disorder, skin ulcer, sweating.

Post Marketing

Post Marketing Adverse Events

The following adverse events have been reported from worldwide marketing experience with Prograf. Because these events are reported voluntarily from a population of uncertain size, are associated with concomitant diseases and multiple drug therapies and surgical procedures, it is not always possible to reliably estimate their frequency or establish a causal relationship to drug exposure. Decisions to include these events in labeling are typically based on one or more of the following factors: (1) seriousness of the event, (2) frequency of the reporting, or (3) strength of causal connection to the drug.

There have been rare spontaneous reports of myocardial hypertrophy associated with clinically manifested ventricular dysfunction in patients receiving Prograf therapy (see PRECAUTIONS-Myocardial Hypertrophy).

Other events include:

Cardiovascular

Atrial fibrillation, atrial flutter, cardiac arrhythmia, cardiac arrest, electrocardiogram T wave abnormal, flushing, myocardial infarction, myocardial ischaemia, pericardial effusion, QT prolongation, Torsade de Pointes, venous thrombosis deep limb, ventricular extrasystoles, ventricular fibrillation

Gastrointestinal

Bile duct stenosis, colitis, enterocolitis, gastroenteritis, gastrooesophageal reflux disease, hepatic cytolysis, hepatic necrosis, hepatotoxicity, impaired gastric emptying, liver fatty, mouth ulceration, pancreatitis haemorrhagic, pancreatitis necrotizing, stomach ulcer, venoocclusive liver disease

Hemic/Lymphatic

Disseminated intravascular coagulation, neutropenia, pancytopenia, thrombocytopenic purpura, thrombotic thrombocytopenic purpura

Metabolic/Nutritional

Glycosuria, increased amylase including pancreatitis, weight decreased

Miscellaneous

Feeling hot and cold, feeling jittery, hot flushes, multi-organ failure, primary graft dysfunction

Nervous System

Carpal tunnel syndrome, cerebral infarction, hemiparesis, leukoencephalopathy, mental disorder, mutism, posterior reversible encephalopathy syndrome (PRES), progressive multifocal leukoencephalopathy (PML), quadriplegia, speech disorder, syncope

Respiratory

Acute respiratory distress syndrome, interstitial lung disease, lung infiltration, respiratory distress, respiratory failure

Skin

Stevens-Johnson syndrome, toxic epidermal necrolysis

Special Senses

Blindness, blindness cortical, hearing loss including deafness, photophobia

Urogenital

Acute renal failure, cystitis haemorrhagic, hemolytic-uremic syndrome, micturition disorder.

OVERDOSAGE

Limited overdosage experience is available. Acute overdosages of up to 30 times the intended dose have been reported. Almost all cases have been asymptomatic and all patients recovered with no sequelae. Occasionally, acute overdosage has been followed by adverse reactions consistent with those listed in the ADVERSE REACTIONS section except in one case where transient urticaria and lethargy were observed. Based on the poor aqueous solubility and extensive erythrocyte and plasma protein binding, it is anticipated that tacrolimus is not dialyzable to any significant extent; there is no experience with charcoal hemoperfusion. The oral use of activated charcoal has been reported in treating acute overdoses, but experience has not been sufficient to warrant recommending its use. General supportive measures and treatment of specific symptoms should be followed in all cases of overdosage.

In acute oral and IV toxicity studies, mortalities were seen at or above the following doses: in adult rats, 52X the recommended human oral dose; in immature rats, 16X the recommended oral dose; and in adult rats, 16X the recommended human IV dose (all based on body surface area corrections).

DOSAGE AND ADMINISTRATION

Prograf injection (tacrolimus injection)

For IV Infusion Only

NOTE: Anaphylactic reactions have occurred with injectables containing castor oil derivatives. See WARNINGS.

In patients unable to take oral Prograf capsules, therapy may be initiated with Prograf injection. The initial dose of Prograf should be administered no sooner than 6 hours after transplantation. The recommended starting dose of Prograf injection is 0.01 mg/kg/day (heart) or 0.03-0.05 mg/kg/day (liver, kidney) as a continuous IV infusion. Adult patients should receive doses at the lower end of the dosing range. Concomitant adrenal corticosteroid therapy is recommended early post-transplantation. Continuous IV infusion of Prograf injection should be continued only until the patient can tolerate oral administration of Prograf capsules.

Preparation for Administration/Stability

Prograf injection must be diluted with 0.9% Sodium Chloride Injection or 5% Dextrose Injection to a concentration between 0.004 mg/mL and 0.02 mg/mL prior to use. Diluted infusion solution should be stored in glass or polyethylene containers and should be discarded after 24 hours. The diluted infusion solution should not be stored in a PVC container due to decreased stability and the potential for extraction of phthalates. In situations where more dilute solutions are utilized (e.g., pediatric dosing, etc.), PVC-free tubing should likewise be used to minimize the potential for significant drug adsorption onto the tubing. Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration, whenever solution and container permit. Due to the chemical instability of tacrolimus in alkaline media, Prograf injection should not be mixed or co-infused with solutions of pH 9 or greater (e.g., ganciclovir or acyclovir).

Prograf capsules (tacrolimus capsules)

Summary of Initial Oral Dosage Recommendations and Observed Whole Blood Trough Concentrations

Patient Population	Recommended Initial Oral Dosage*	Observed Whole Blood Trough Concentrations
Adult kidney transplant patients		
In combination with azathioprine	0.2 mg/kg/day	month 1-3: 7-20 ng/mL month 4-12: 5-15 ng/mL
In combination with MMF/IL-2 receptor antagonist **	0.1 mg/kg/day	month 1-12: 4-11 ng/mL

Adult liver transplant patients	0.10-0.15 mg/kg/day	month 1-12: 5-20 ng/mL
Pediatric liver transplant patients	0.15-0.20 mg/kg/day	month 1-12: 5-20 ng/mL
Adult heart transplant patients	0.075 mg/kg/day	month 1-3: 10-20 ng/mL month ≥4: 5-15 ng/mL

* Note : two divided doses, q12h

** In a second smaller study, the initial dose of tacrolimus was 0.15-0.2 mg/kg/day and observed tacrolimus concentrations were 6-16 ng/mL during month 1-3 and 5-12 ng/mL during month 4-12 (see CLINICAL STUDIES).

Liver Transplantation

It is recommended that patients initiate oral therapy with Prograf capsules if possible. If IV therapy is necessary, conversion from IV to oral Prograf is recommended as soon as oral therapy can be tolerated. This usually occurs within 2-3 days. The initial dose of Prograf should be administered no sooner than 6 hours after transplantation. In a patient receiving an IV infusion, the first dose of oral therapy should be given 8-12 hours after discontinuing the IV infusion. The recommended starting oral dose of Prograf capsules is 0.10 to 0.15 mg/kg/day administered in two divided daily doses every 12 hours. Co-administered grapefruit juice has been reported to increase tacrolimus blood trough concentrations in liver transplant patients. (See Drugs that May Alter Tacrolimus Concentrations).

Dosing should be titrated based on clinical assessments of rejection and tolerability. Lower Prograf dosages may be sufficient as maintenance therapy. Adjunct therapy with adrenal corticosteroids is recommended early post-transplant.

Dosage and typical tacrolimus whole blood trough concentrations are shown in the table above; blood concentration details are described in Blood Concentration Monitoring: *Liver Transplantation* below.

Kidney Transplantation

The recommended starting oral dose of Prograf (administered every 12 hours in two divided doses) is 0.2 mg/kg/day when used in combination with azathioprine or 0.1 mg/kg/day when used in combination with MMF and IL-2 receptor antagonist (see CLINICAL STUDIES). The initial dose of Prograf may be administered within 24 hours of transplantation, but should be delayed until renal function has recovered (as indicated for example by a serum creatinine ≤ 4 mg/dL). Black patients may require higher doses to achieve comparable blood concentrations. Dosage and typical tacrolimus whole blood trough concentrations are shown in the table above; blood concentration details are described in Blood Concentration Monitoring: *Kidney Transplantation* below.

The data in kidney transplant patients indicate that the Black patients required a higher dose to attain comparable trough concentrations compared to Caucasian patients.

Time After Transplant	**Caucasian n=114**		**Black n=56**	
	Dose (mg/kg)	**Trough Concentrations (ng/mL)**	**Dose (mg/kg)**	**Trough Concentrations (ng/mL)**

Time After Transplant	Caucasian n=114		Black n=56	
	Dose (mg/kg)	Trough Concentrations (ng/mL)	Dose (mg/kg)	Trough Concentrations (ng/mL)
Day 7	0.18	12.0	0.23	10.9
Month 1	0.17	12.8	0.26	12.9
Month 6	0.14	11.8	0.24	11.5
Month 12	0.13	10.1	0.19	11.0

Heart Transplantation

The recommended starting oral dose of Prograf is 0.075 mg/kg/day administered every 12 hours in two divided doses. If possible, initiating oral therapy with Prograf capsules is recommended. If IV therapy is necessary, conversion from IV to oral Prograf is recommended as soon as oral therapy can be tolerated. This usually occurs within 2-3 days. The initial dose of Prograf should be administered no sooner than 6 hours after transplantation. In a patient receiving an IV infusion, the first dose of oral therapy should be given 8-12 hours after discontinuing the IV infusion.

Dosing should be titrated based on clinical assessments of rejection and tolerability. Lower Prograf dosages may be sufficient as maintenance therapy. Adjunct therapy with adrenal corticosteroids is recommended early post transplant.

Dosage and typical tacrolimus whole blood trough concentrations are shown in the table above; blood concentration details are described in Blood Concentration Monitoring: *Heart Transplantation* below.

Pediatric Patients

Pediatric liver transplantation patients without pre-existing renal or hepatic dysfunction have required and tolerated higher doses than adults to achieve similar blood concentrations. Therefore, it is recommended that therapy be initiated in pediatric patients at a starting IV dose of 0.03-0.05 mg/kg/day and a starting oral dose of 0.15-0.20 mg/kg/day. Dose adjustments may be required. Experience in pediatric kidney and heart transplantation patients is limited.

Patients with Hepatic or Renal Dysfunction

Due to the reduced clearance and prolonged half-life, patients with severe hepatic impairment (Pugh $\geq$ 10) may require lower doses of Prograf. Close monitoring of blood concentrations is warranted.

Due to the potential for nephrotoxicity, patients with renal or hepatic impairment should receive doses at the lowest value of the recommended IV and oral dosing ranges. Further reductions in dose below these ranges may be required. Prograf therapy usually should be delayed up to 48 hours or longer in patients with post-operative oliguria.

Conversion from One Immunosuppressive Regimen to Another

Prograf should not be used simultaneously with cyclosporine. Prograf or cyclosporine should be discontinued at least 24 hours before initiating the other. In the presence of elevated Prograf or cyclosporine concentrations, dosing with the other drug usually should be further delayed.

Blood Concentration Monitoring

Monitoring of tacrolimus blood concentrations in conjunction with other laboratory and clinical parameters is considered an essential aid to patient management for the evaluation of rejection, toxicity, dose adjustments and compliance. Factors influencing frequency of monitoring include but are not limited to hepatic or renal dysfunction, the addition or discontinuation of potentially interacting drugs and the posttransplant time. Blood concentration monitoring is not a replacement for renal and liver function monitoring and tissue biopsies.

Two methods have been used for the assay of tacrolimus, a microparticle enzyme immunoassay (MEIA) and ELISA. Both methods have the same monoclonal antibody for tacrolimus. Comparison of the concentrations in published literature to patient concentrations using the current assays must be made with detailed knowledge of the assay methods and biological matrices employed. Whole blood is the matrix of choice and specimens should be collected into tubes containing ethylene diamine tetraacetic acid (EDTA) anti-coagulant. Heparin anti-coagulation is not recommended because of the tendency to form clots on storage. Samples which are not analyzed immediately should be stored at room temperature or in a refrigerator and assayed within 7 days; if samples are to be kept longer they should be deep frozen at -20° C for up to 12 months.

Liver Transplantation

Although there is a lack of direct correlation between tacrolimus concentrations and drug efficacy, data from Phase II and III studies of liver transplant patients have shown an increasing incidence of adverse events with increasing trough blood concentrations. Most patients are stable when trough whole blood concentrations are maintained between 5 to 20 ng/mL. Long-term post-transplant patients often are maintained at the low end of this target range.

Data from the U.S. clinical trial show that tacrolimus whole blood concentrations, as measured by ELISA, were most variable during the first week post-transplantation. After this early period, the median trough blood concentrations, measured at intervals from the second week to one year post-transplantation, ranged from 9.8 ng/mL to 19.4 ng/mL.

Therapeutic Drug Monitoring, 1995, Volume 17, Number 6 contains a consensus document and several position papers regarding the therapeutic monitoring of tacrolimus from the 1995 International Consensus Conference on Immunosuppressive Drugs. Refer to these manuscripts for further discussions of tacrolimus monitoring.

Kidney Transplantation

Data from a Phase 3 study of Prograf in conjunction with azathioprine indicate that trough concentrations of tacrolimus in whole blood, as measured by IMx® were most variable during the first week of dosing. During the first three months of that trial, 80% of the patients maintained trough concentrations between 7-20 ng/mL, and then between 5-15 ng/mL, through 1 year.

In a separate clinical trial of Prograf in conjunction with MMF and daclizumab, approximately 80% of patients maintained tacrolimus whole blood concentrations between 4-11 ng/mL through 1 year post-transplant.

In another clinical trial of Prograf in conjunction with MMF and basiliximab, approximately 80% of patients maintained tacrolimus whole trough blood concentrations between 6-16 ng/mL during month 1-3 and, then, between 5-12 ng/mL from month 4 through 1 year.

The relative risks of toxicity and efficacy failure are related to tacrolimus whole blood trough concentrations. Therefore, monitoring of whole blood trough concentrations is recommended to assist in the clinical evaluation of toxicity and efficacy failure.

Heart Transplantation

Data from a European Phase 3 study indicate that trough concentrations of tacrolimus in whole blood, as measured by IMx® were most variable during the first week of dosing. From 1 week to 3 months post transplant, approximately 80% of patients maintained trough concentrations between 8-20 ng/mL and, from 3 months through 18 months post transplant, approximately 80% of patients maintained trough concentrations between 6-18 ng/mL.

The relative risk of toxicity; for example, nephrotoxicity and post-transplant diabetes mellitus, is increased with higher trough concentrations. Therefore, monitoring of whole blood trough concentrations is recommended to assist in the clinical evaluation of toxicity.

HOW SUPPLIED

Prograf capsules (tacrolimus capsules)

strength	**0.5 mg** (containing the equivalent of 0.5 mg anhydrous tacrolimus)	**1 mg** (containing the equivalent of 1 mg anhydrous tacrolimus)	**5 mg** (containing the equivalent of 5 mg anhydrous tacrolimus)
shape/color	oblong/light yellow	oblong/white	oblong/grayish red
branding on capsule cap/body	f 607	f 617	f 657
100 count bottle **10 blister cards of 10 capsules**	**NDC 0469-0607-73**	**NDC 0469-0617-73** **NDC 0469-0617-11**	**NDC 0469-0657-73** **NDC 0469-0657-11**

Made in Japan

Store and Dispense

Store at 25°C (77°F); excursions permitted to 15°C-30°C (59°F-86°F).

Prograf injection (tacrolimus injection)

(for IV infusion only)

NDC 0469-3016-01 Product Code 301601

5 mg/mL (equivalent of 5 mg of anhydrous tacrolimus per mL) supplied as a sterile solution in a 1 mL ampule, in boxes of 10 ampules

Made in Ireland

Store and Dispense

Store between 5°C and 25°C (41°F and 77°F).

Rx only

Marketed by:

Astellas Pharma US, Inc.

Deerfield, IL 60015-2548

Revised: August 2009

09H011-PRG-WPI

REFERENCE

1.CDC: Recommendations of the Advisory Committee on Immunization Practices: Use of vaccines and immune globulins in persons with altered immunocompetence. MMWR 1993;42(RR-4):1-18.